sustainability

MDPI

Article

Physiological and Biochemical Behaviours and Antioxidant Response of *Helianthus annuus* under Lanthanum and Cerium Stress

Nesrine Dridi [1], Pedro Brito [2,3], Houda Bouslimi [1], Renata Ferreira [4], Susete Martins-Dias [5], Isabel Caçador [6] and Noomene Sleimi [1,*]

[1] Research Laboratory (LR)—Resources, Materials and Ecosystems (RME), Faculty of Sciences of Bizerte, University of Carthage, Bizerte 7021, Tunisia; nesrinedridi442@yahoo.fr (N.D.); houda0702@hotmail.com (H.B.)
[2] Instituto Português do Mar e da Atmosfera (IPMA), Division of Oceanography and Marine Environment, Av. Alfredo Magalhães Ramalho, 6, 1495-165 Algés, Portugal; pbrito@ipma.pt
[3] Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Avenida General Norton de Matos S/N, 4450-208 Matosinhos, Portugal
[4] Centro de Recursos Naturais e Ambiente (CERENA), Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; renata.ferreira@tecnico.ulisboa.pt
[5] Centro de Recursos Naturais e Ambiente (CERENA), Departamento de Bioengenharia (DBE), Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal; susetedias@tecnico.ulisboa.pt
[6] Centro de Ciências do Mar e do Ambiente (MARE), Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal; micacador@fc.ul.pt
* Correspondence: noomene.sleimi@gmail.com

Abstract: The continuous progress of global manufacturing and anthropogenic activities has resulted in excessive environmental metallic pollution, particularly with rare earth elements (REEs) which have become a prevalent issue of global concern due to their high toxicity and widespread existence. REEs-contaminated soils could ruin agriculture by inducing plant physiology disturbances in various crops that are considered the principal link of the human food chain. The main purpose of the present work is to assess the phytotoxicity of two light REEs, lanthanum (La) and cerium (Ce), in *Helianthus annuus* after 14 days of exposure to different concentrations of La and Ce (0, 1, 2.5, 5, and 10 µM). Plants showed different variations in shoot and root lengths at the end of the trial period. The accumulation of photosynthetic pigments, such as chlorophylls and carotenoids, as well as the photosynthetic efficiency, the non-photochemical quenching, the photosynthetically active radiation, and the electron transport rate, increased in the two REE treatments. Hydrogen peroxide significantly increased in all applied concentrations of La and Ce. A significant increase in malondialdehyde content was noticed only when plants were exposed to 2.5 µM La and 10 µM Ce. Results also demonstrated that La and Ce induced an increase in the activity of superoxide dismutase, peroxidase, and catalase (only the highest concentration of La decreased catalase activity). The exposure to different REE concentrations induced the accumulation of La and Ce in the plants, mainly in roots. *Helianthus annuus* showed an effective resistance behaviour facing La- and Ce-induced stresses.

Keywords: lanthanum; cerium; antioxidant enzymes; photosynthetic pigments; chlorophyll fluorescence

Citation: Dridi, N.; Brito, P.; Bouslimi, H.; Ferreira, R.; Martins-Dias, S.; Caçador, I.; Sleimi, N. Physiological and Biochemical Behaviours and Antioxidant Response of *Helianthus annuus* under Lanthanum and Cerium Stress. *Sustainability* **2022**, *14*, 4153. https://doi.org/10.3390/su14074153

Academic Editor: Adriano Sofo

Received: 22 February 2022
Accepted: 28 March 2022
Published: 31 March 2022

1. Introduction

Lanthanum (La) and cerium (Ce) are two of the 15 lanthanides of the rare earth elements (REEs) group which are naturally and abundantly existent in the Earth's crust [1–3], where the average abundance is 35 µg g^{-1} in La and 66 µg g^{-1} in Ce [4]. REEs are widely used in new developed technologies, including electronics, chemical engineering, the

electronic aerospace industry, renewable energy, medical sciences, and in agricultural fertilisers [5,6].

In fact, crop quality and yield might be improved when an appropriate amount of La and Ce is applied due to the similarity of the chemical properties of these REEs to macronutrients such potassium, calcium, and magnesium [7–9]. Moreover, the photosynthetic rate can also be promoted in plants when exposed to REEs [3]. Previous examples have shown the efficiency of La and Ce in plants photosynthesis amelioration, where a concentration of 25 and 50 µM La increased the chlorophyll index and the photosynthetic rate in maize [9], and chlorophyll a and photosynthetic rate were also enhanced in *Glycine max* in 100 and 500 $mg.kg^{-1}$ Ce treatments [10]. Furthermore, the application of varying concentrations of La (from 5 to 35 µM) on horseradish plants increased net photosynthetic rate and chlorophyll content [11]. The chlorophyll levels also increased in rice treated with 0.05 and 0.1 mM Ce [12].

However, the excessive use of REEs may results in an increase of reactive oxygen species (ROS) accumulation in the plant cells inducing an oxidative stress at high concentrations. This oxidative stress can lead to an imbalance in plant metabolism, resulting in an inhibition of plant growth and photosynthesis [3,13,14]. D'Aquino et al. [15] reported the negative effect of La (III) treatments (from 0.01 to 10 mM La) on *Triticum durum* that showed a significant reduction in root and shoot lengths. The addition of 0.5–25 mg L^{-1} of La or Ce to the growth medium decreased root and shoot elongation and root dry biomass of *Triticum aestivum* seedlings [16]. In addition, chlorophyll a and b contents and the net photosynthetic rate (*Pn*) decreased in rice exposed to 0.08 and 2.4 mM La, and the reported decline was due to the damage that this REE induced on the ultrastructure of the chloroplast [17].

To protect the plant from the oxidative damage, the antioxidant enzymatic system, which includes enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), is rapidly activated and involved in ROS detoxification [18,19]. In plant cells, SOD is a crucial oxidative defender allowing the adaptation of plants to various stressed environments [20,21], whereas POD and CAT are responsible for the reduction of the toxic hydrogen peroxide (H_2O_2) produced during SOD action [22,23]. Kovaříková et al. [24] reported that the application of REE treatments on cucumber and rice increased both POD and SOD activity, while CAT was enhanced in rice species.

The main objective of this work was to evaluate the response of *Helianthus annuus* plants to the stress induced by exposure to different concentrations of La and Ce through: (1) the evaluation of chlorophyll fluorescence and the content of chlorophyll a and b and carotenoids; (2) the study of the cell damage degree, by determining the levels of H_2O_2 and MDA; (3) the evaluation of antioxidant activity of the SOD, CAT, and POD enzymes, and (4) the assessment of the susceptibility of potential accumulation of REEs in plant organs.

2. Materials and Methods

2.1. Plant Culture and REEs Treatment

Helianthus annuus seeds were harvested from sunflower farms located in Béja in the north of Tunisia (36°49′90″ N, 9°13′77″ E). The species has been identified by Wunderlin and the plant specimen was presented in the herbarium of the Atlas of Florida (Accession number: 134009).

Growth and treatment experiments of *Helianthus annuus* plants were performed in a hydroponic system in a controlled chamber (Fitoclima S600, Aralab, Rio de Mouro, Portugal) with a photoperiod of 12/12 h day/night, at a mean temperature of 25 ± 5 °C, and a relative humidity range of 60–80%. *Helianthus annuus* seeds were sterilised in hypochlorite calcium solution (5%) and rinsed and soaked in distilled water for 2 h. Subsequently, seeds were settled in Petri dishes coated by a double layer of filter paper moisturised with distilled water and placed in the dark for five days to germinate. After 15 days of sowing, developed seedlings were irrigated, five days before starting the REE treatments, with 1/4 Hoagland nutrient solutions.

Afterwards, five groups of 15 uniform plants (20 days old) were treated separately with five prepared concentrations of La or Ce (0, 1, 2.5, 5, and 10 µM), for 14 days. For the REE exposure, the Hoagland's growth nutritive solution was supplied with La and Ce prepared from 1000 mg La/L and 1000 mg Ce/L certified reference standard solutions with high purity of La and Ce (Sigma Aldrich, St. Louis, MO, USA).

At the end of the exposure period, plants were collected and the lengths of shoots and roots were measured. Leaf tissues were immediately ground and stored in liquid nitrogen. Leaves are the main responsible organ of all biochemical and physiological reactions in the plants, and due to the reduced quantities of *Helianthus annuus* roots obtained, the assessment of enzymatic activity, lipid peroxidation, and hydrogen peroxide level was carried out only in the leaves.

2.2. Fluorescence Measurement

Modulated chlorophyll a fluorescence measurements were performed in *Helianthus annuus* leaves using a MINI-PAM Portable Chlorophyll Fluorometer (Heinz Walz GmbH, Germany). The maximal photochemical yield (Fv/Fm) and the photochemical efficiency (Fv'/Fm') of photosystem-II (PSII), as well as the non-photochemical quenching coefficient (NPQ), were determined [6,25,26]. The electron transport rate (ETR) and the photosynthetically active radiation (PAR) were also measured.

Measurements were taken for all the plants of each treatment groups (15 plants/treatment) and the average of 15 different values was calculated for each studied parameter, while the leaf clip position was randomly selected.

2.3. Photosynthetic Pigments Content

The photosynthetic pigments chlorophyll a (Chl a), chlorophyll b (Chl b), and total carotenoids (Car) were extracted by mixing 25 mg of ground leaf tissues with 4 mL of ethanol 95% for 2 h at room temperature and in the dark. After the 2 h incubation period, the extract was centrifuged for 10 min at 4000 rpm [27]. The absorbance of the obtained supernatant was measured at 470, 648.6, and 664.2 nm and pigment quantifications were determined following Lichtenthaler [28].

2.4. Endogenous Hydrogen Peroxide Content

The H_2O_2 content was determined in leaves following Bouazizi et al. [29], by homogenising 250 mg of the fresh ground tissue with 1.5 mL of 0.1% of trichloroacetic acid solution (TCA) on an ice bath. The mixture was centrifuged at 12,000× *g* at 4 °C for 15 min. A volume of 0.5 mL of the resulted supernatant was added to 0.5 mL of potassium phosphate buffer (10 mM, pH 7) and 1 mL of potassium iodide (1 M) and the absorbance was determined at 390 nm.

2.5. Lipid Peroxidation

Lipid peroxidation degree was assessed by the determination of the produced malondialdehyde (MDA) in fresh leaf tissues. MDA levels were quantified according to Velikova and Loreto's method [30] with slight changes. Briefly, 100 mg of ground material was homogenised in 1 mL of 0.1% trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 12,000× *g* for 15 min and 0.5 mL of the obtained supernatant was added to 1 mL 0.5% (*w*/*v*) thiobarbituric acid in 20% TCA. Afterwards, the mixture was incubated at 100 °C for 30 min. The reaction was stopped by transferring the reaction tubes to an ice bath. Finally, the samples were centrifuged at 10,000× *g* for 5 min and the absorbance of the supernatant was measured at 532 nm and 600 nm.

2.6. Protein Crude Extract Preparation and Enzymatic Activity Determination

Helianthus annuus leaves crude extracts were prepared following Davis and Swanson [31] protocol and Ferreira et al. [27] optimisation. Total protein concentrations determi-

nations were performed with Pierce® BCA protein assay kit using bovine serum albumin (BSA) as standard.

SOD (EC 1.15.1.1) and CAT (EC 1.11.1.6) activities were assessed according to the reported methods in Brito et al. [32], while the determination of POD (EC 1.11.1.7) activity was carried out as described in Ferreira and Martins-Dias [33].

2.7. Determination of REEs Contents

The REEs accumulation in *Helianthus annuus* plants was assessed, after the digestion of the samples as described in Labidi et al. [34] and Sleimi et al. [35], by the determination of La and Ce contents in the shoots and roots following the described method in Brito et al. [32].

2.8. Statistical Analysis

Each treatment was performed in three biological replicates (five plants/replicate) and each replicate was analysed in triplicate (n = 9). Values were expressed as means ± standard deviation (SD) and results were considered significantly different at $p < 0.05$, using one-way ANOVA analysis and Tukey's honest significant difference (HSD) test. Principal component analysis (PCA) was performed using R software V 4.1.3.

3. Results

3.1. Plant Growth

Lanthanum and cerium stresses differently affected the lengths of shoots and roots of the *Helianthus annuus* plants measured at the end of the experiment exposure (Figure 1). No significant variation was noted in the shoot lengths of La-treated plants ($p > 0.05$). A similar trend was observed in the roots, except in the roots of 10 µM La, which showed a significant increase as compared to the control ($p < 0.05$). Conversely, results showed that Ce treatment only induced the increase of shoots length at 2.5 and 5 µM concentrations ($p < 0.05$), and enhanced roots length in 1 and 10 µM treated plants ($p < 0.05$). The length of roots in 2.5 µM Ce significantly declined in comparison with the untreated plants ($p < 0.05$).

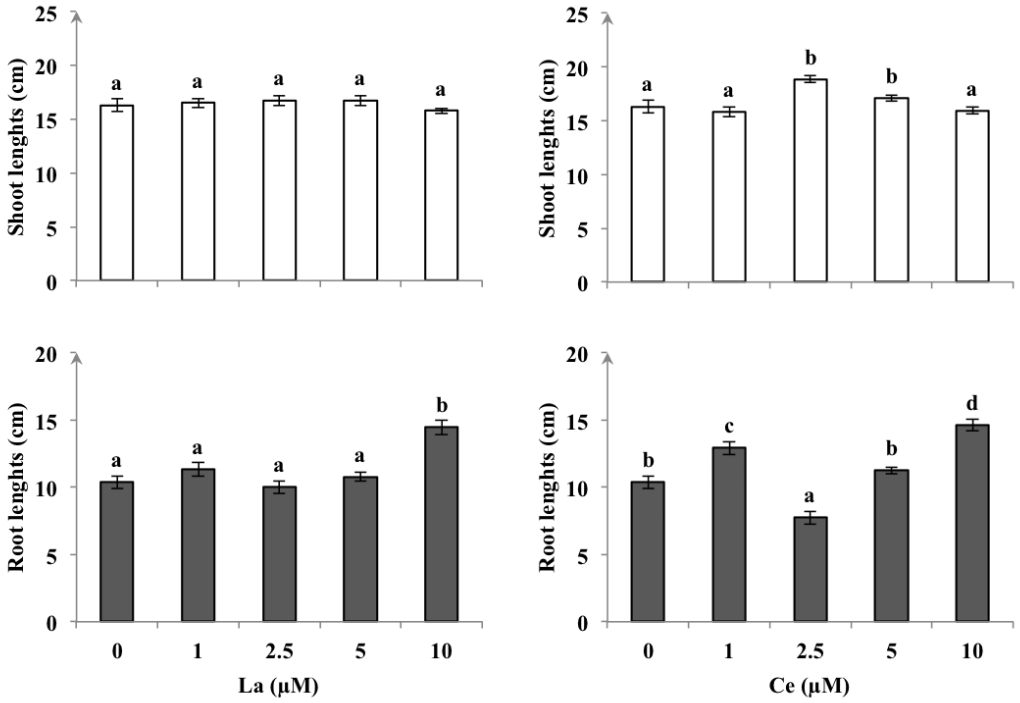

Figure 1. Shoot and root lengths in La- and Ce-treated *Helianthus annuus* plants after 14 days of REE exposure (values are means ± SD, n = 9). Within the plant tissue and treatment group, values with different letters were significantly different at $p < 0.05$.

Overall, the observation of La- and Ce-treated plants of *Helianthus annuus* species, at the end of metal exposure, showed that the two REEs did not harmfully affect the plants (Figure 2).

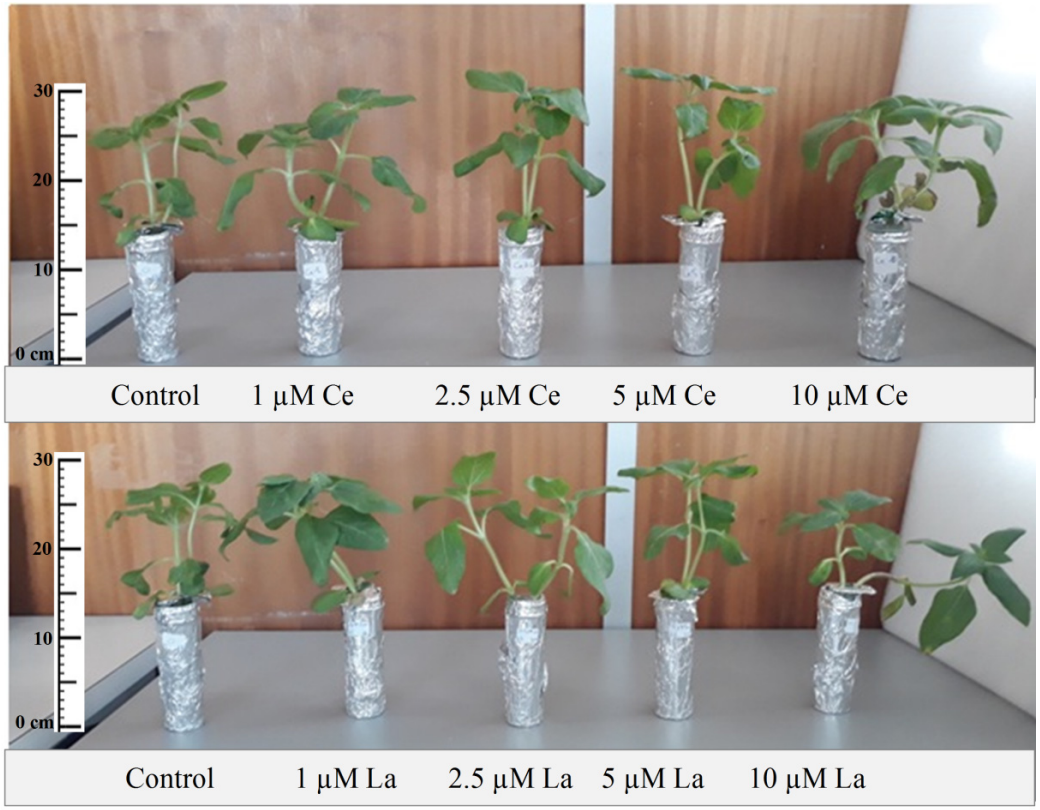

Figure 2. An example showing the morphology of *Helianthus annuus* plants at the end of lanthanum (bottom picture) and cerium (top picture) treatments.

3.2. Chlorophyll Fluorescence Response

Results in Table 1 show that the maximum quantum yield of PSII (Fv/Fm) increased in *Helianthus annuus* plants under the effects of La and Ce stress when compared to the control. Nevertheless, the increase was only significant in 10 µM La and in 2.5 and 10 µM Ce treatments ($p < 0.05$). Moreover, the photochemical efficiency of PSII in the light (Fv'/Fm') in exposed plants was statistically similar to the untreated plants; a slight increase was observed in all La and Ce treatments without any notable significant difference compared to the control ($p > 0.05$).

In comparison to the control, the non-photochemical quenching (NPQ) was affected by the two studied REEs (Table 1). This parameter was significantly elevated in 1, 5, and 10 µM La treatments, reaching 0.94, 1.05, and 1.14, respectively. Significant increases were also noticed in plants treated with 2.5, 5, and 10 µM Ce concentrations and the NPQ rate reached 1.18, 1.19, and 1.06, respectively ($p < 0.01$).

The electron transport rate (ETR) and the photosynthetically active radiation (PAR) followed the same trends and were found significantly higher ($p < 0.01$) than the control. The obtained rates were approximately doubled in all La-treated plants (except 10 µM dose; Table 1). ETR and PAR were also significantly enhanced in all Ce treatments (Table 1).

Principal Component Analysis

Statistical analysis was performed using PCA to study the influence of REEs on the photosynthetic quenching parameters and to assess the correlation between the determined parameters under the effects of La and Ce (Figure 3). PCA revealed a clear separation between the control and the lower concentrations of La treatments (1 and 2.5 µM) (Figure 3A),

and the separation was clear only between the control and the concentration of 5 µM Ce (Figure 3B). Concerning the La conditions, there was a clear separation between scores of the lowest La concentrations (1 and 2.5 µM) and the highest one (10 µM), whereas the separation was not clear for all Ce groups. In La treatment, Fv/Fm and NPQ were positively correlated; a significant positive correlation was also observed between PAR and ETR (Figure 3A). Results also showed positive correlation between NPQ and PAR in Ce (Figure 3B).

Table 1. Chlorophyll fluorescence parameters and pigments content ratios in *Helianthus annuus* exposed to La and Ce during 14 days of treatment.

Treatment	Fv/Fm	Fv′/Fm′	NPQ	ETR	PAR	Chl a/Chl b	(Chl a + Chl b)/Car
La (µM)							
0	$0.64^{a} \pm 0.02$	$0.83^{a} \pm 0.01$	$0.73^{a} \pm 0.02$	$0.70^{a} \pm 0.00$	$2.0^{a} \pm 0.00$	2.80 ± 0.07	5.14 ± 0.06
1	$0.70^{ab} \pm 0.02$	$0.85^{a} \pm 0.01$	$0.94^{b} \pm 0.06$	$1.35^{b} \pm 0.04$	$3.53^{b} \pm 0.13$	3.05 ± 0.04	5.00 ± 0.05
2.5	$0.69^{ab} \pm 0.01$	$0.86^{a} \pm 0.01$	$0.76^{a} \pm 0.04$	$1.45^{b} \pm 0.06$	$4.0^{c} \pm 0.17$	2.87 ± 0.03	5.24 ± 0.06
5	$0.71^{ab} \pm 0.02$	$0.88^{a} \pm 0.01$	$1.05^{b} \pm 0.05$	$1.35^{b} \pm 0.09$	$3.53^{b} \pm 0.26$	2.94 ± 0.08	5.12 ± 0.05
10	$0.74^{b} \pm 0.02$	$0.84^{a} \pm 0.01$	$1.14^{b} \pm 0.06$	$0.70^{a} \pm 0.00$	$2.0^{a} \pm 0.00$	2.98 ± 0.03	5.24 ± 0.07
Ce (µM)							
0	$0.64^{a} \pm 0.02$	$0.83^{a} \pm 0.01$	$0.73^{a} \pm 0.02$	$0.70^{a} \pm 0.00$	$2.0^{a} \pm 0.00$	2.80 ± 0.07	5.14 ± 0.06
1	$0.72^{ab} \pm 0.02$	$0.88^{a} \pm 0.01$	$0.75^{a} \pm 0.03$	$1.38^{c} \pm 0.06$	$4.0^{c} \pm 0.2$	3.08 ± 0.04	5.01 ± 0.03
2.5	$0.79^{b} \pm 0.02$	$0.86^{a} \pm 0.01$	$1.18^{b} \pm 0.04$	$1.19^{b} \pm 0.07$	$2.93^{b} \pm 0.18$	2.91 ± 0.03	5.21 ± 0.06
5	$0.70^{ab} \pm 0.02$	$0.85^{a} \pm 0.01$	$1.19^{b} \pm 0.04$	$1.12^{b} \pm 0.09$	$2.93^{b} \pm 0.21$	3.07 ± 0.08	5.07 ± 0.09
10	$0.73^{b} \pm 0.01$	$0.84^{a} \pm 0.01$	$1.06^{b} \pm 0.03$	$0.97^{b} \pm 0.06$	$2.53^{b} \pm 0.13$	2.94 ± 0.03	4.99 ± 0.06

Fv/Fm: maximum PSII photochemical efficiency, Fv′/Fm′: photochemical efficiency of PSII in the light, NPQ: non-photochemical quenching, PAR: photosynthetically active radiation, ETR: electron transport rate, Chl: chlorophyll, Car: carotenoids. Values are means ± SD. Values with different lower-case letters are significantly different at $p < 0.05$, n = 15.

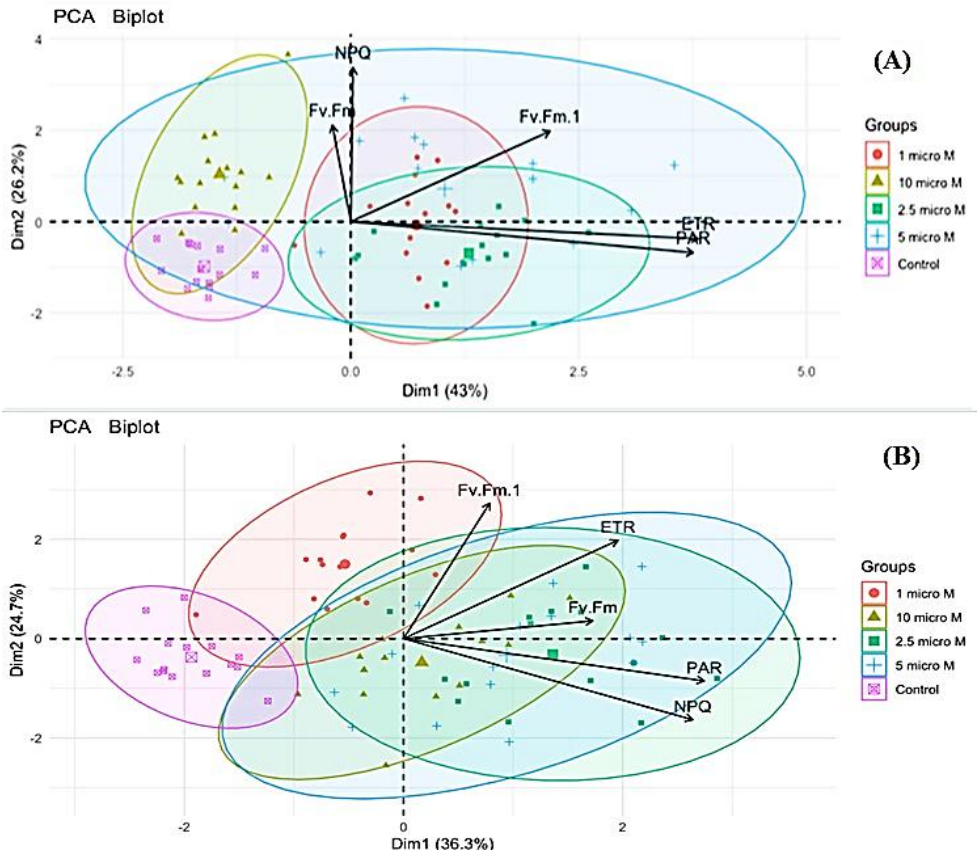

Figure 3. Principal component analysis (PCA) biplot (x: first component, y: second component) of

the chlorophyll fluorescence data of *Helianthus annuus* leaves under the effect of La (**A**) and Ce (**B**). PC1 explained 43% and 36.3% of the variance in La and Ce treatments, respectively, while PC2 explained only 26.2% and 24.7% of the variance in La and Ce treatments. Different colours depict the concentrations of supplied La or Ce in the growth medium, while arrows show gradients resulting from the influence of REEs on the photosynthetic quenching parameters. Fv.Fm corresponds to Fv/Fm: maximum PSII photochemical efficiency, Fv.Fm1 corresponds to Fv'/Fm': photochemical efficiency of PSII in the light, NPQ: non-photochemical quenching, PAR: photosynthetically active radiation, ETR: electron transport rate.

3.3. Photosynthetic Pigments Contents

As presented in Figure 4, La and Ce treatments resulted in a significant variation in pigment content in treated *Helianthus annuus* plants, essentially in the highest concentrations. Lanthanum exposure induced a significant increase in Chl a ($p < 0.001$), Chl b ($p < 0.05$), and carotenoid ($p < 0.001$) levels (by 19%, 12%, and 17% in 5 µM La and by 22%, 14%, and 18% in 10 µM La, respectively, as compared to the control). No significant difference was noted in 1 and 2.5 µM La. However, in comparison to the untreated plants, the exposure of the plants to 2.5 and 10 µM Ce also caused a significant enhancement in Chl a (up to 20% and 27%, respectively), in Chl b (up to 15% and 20%, respectively), and in carotenoids (up to 17% and 29%, respectively).

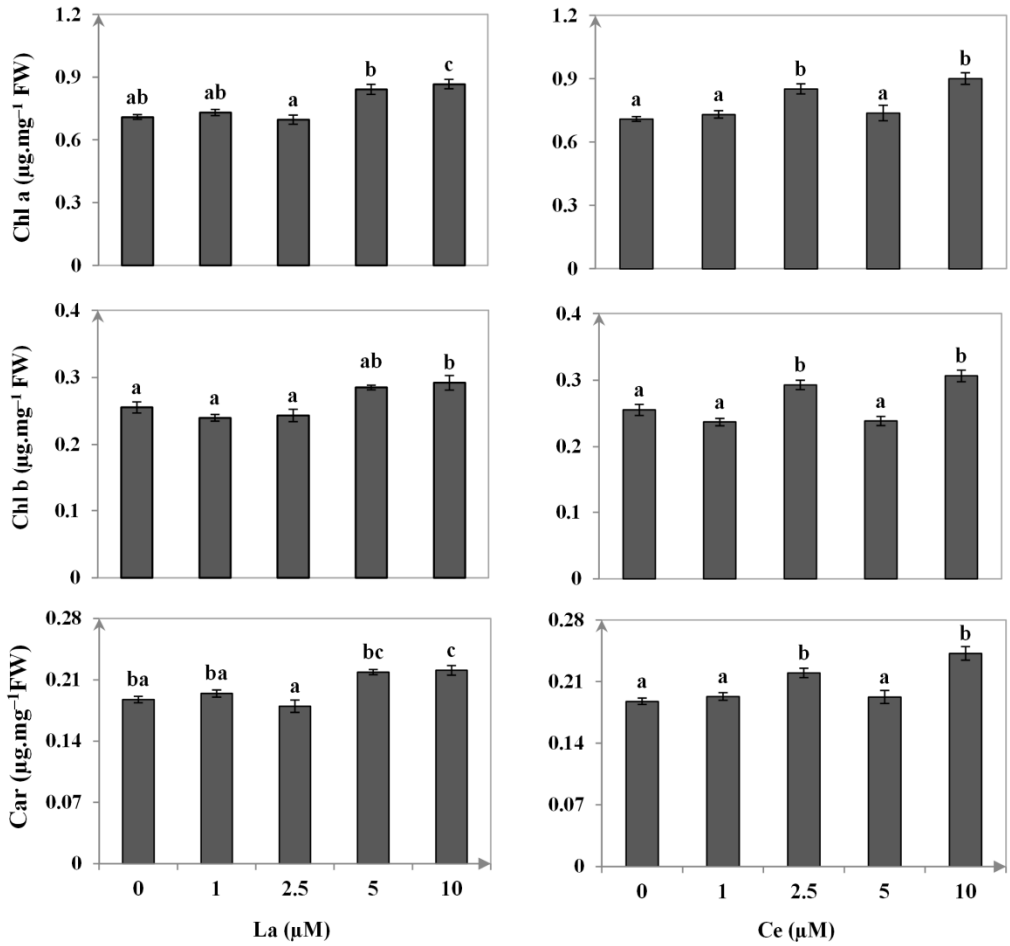

Figure 4. Chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (Car) content in the leaves of *Helianthus annuus* plants exposed to La (on the left side) and Ce (on the right side) over 14 days of treatments (values are means ± SD, n = 9). Within a given parameter, values with lower-case letters were significantly different at $p < 0.05$, respectively.

3.4. Endogenous H_2O_2 Content and Lipid Peroxidation

Lanthanum- and cerium-induced stresses significantly increased the endogenous H_2O_2 content ($p < 0.01$) by more than 3.3-fold in *Helianthus annuus* plants treated with all concentrations, as compared to the control (Figure 5).

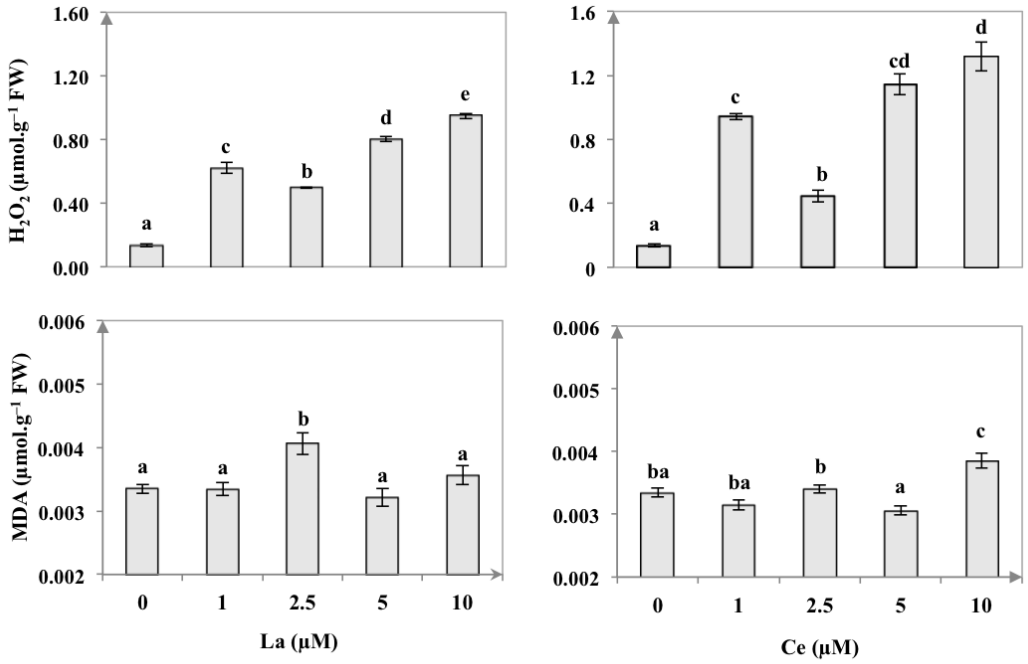

Figure 5. Content of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in the leaves of *Helianthus annuus* after 14 days of exposure to different La (on the left side) and Ce (on the right side) concentrations (values are means ± SD, n = 9). Values with different letters were significantly different at $p < 0.01$ and $p < 0.05$.

As shown in Figure 5, the MDA levels were only significantly increased in the 2.5 µM La and 10 µM Ce treatment groups ($p < 0.05$), whereas at the other tested concentrations of La and Ce, no significant variation was observed when compared to the untreated plants ($p > 0.05$).

3.5. Enzymatic Activity

The application of La and Ce treatments on *Helianthus annuus* plants triggered a notable and significant increase in plant leaves SOD activity ($p < 0.01$), with more than 2-fold enhancement observed in all treatments as compared to the control (Figure 6).

Moreover, when compared to the control, POD activity increased in all La and Ce tested concentrations ($p < 0.01$) (Figure 6).

Regarding CAT activity (Figure 6), a significant increase in 1, 2.5, and 5 µM La and in 2.5, 5, and 10 µM Ce treatments was noted in comparison to the untreated plants ($p < 0.01$). The lowest concentration of Ce (1 µM) did not show any significant variation in this enzyme activity in plant leaves when compared to the control. However, 10 µM La significantly decreased CAT activity in the studied plant (Figure 6).

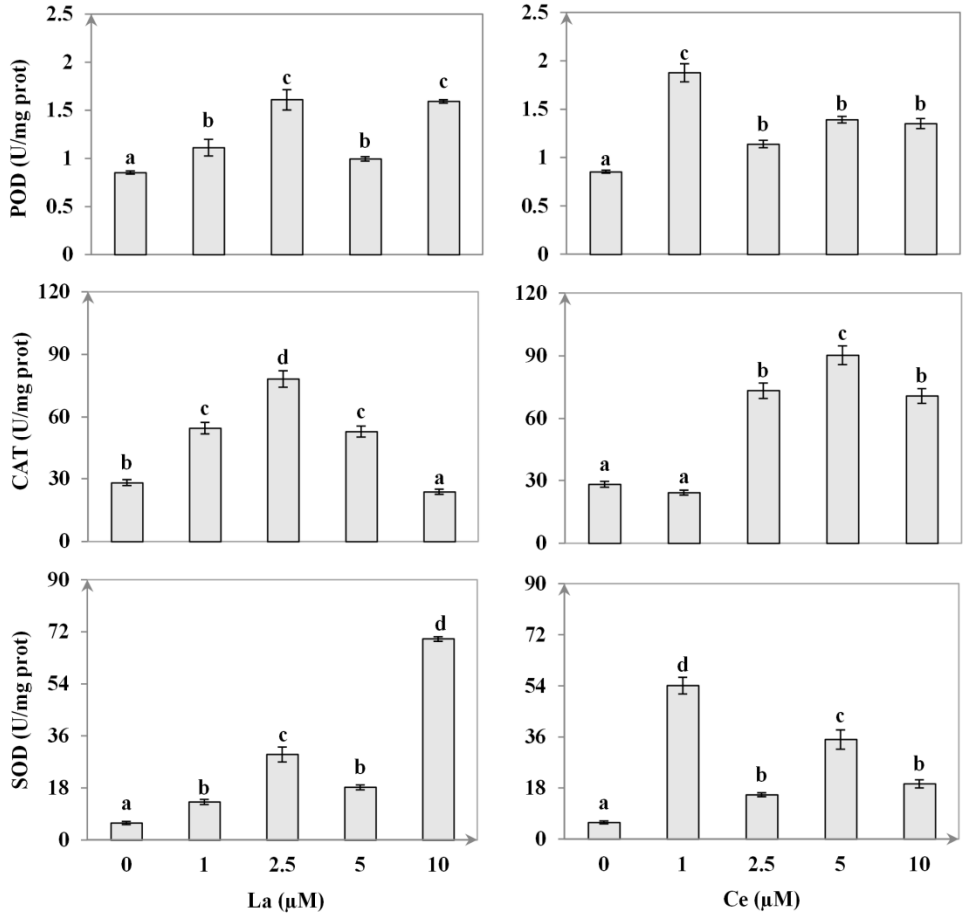

Figure 6. Antioxidant activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in *Helianthus annuus* leaves after 14 days of exposure to different La (on the left side) and Ce (on the right side) concentrations (values are means ± SD, n = 9). Within a given parameter, values with different letters were significantly different at $p < 0.01$.

3.6. REEs Contents

Results presented in Figure 7 showed that *Helianthus annuus* was capable of accumulating La and Ce in the studied plant organs, even though in varying proportions depending on the tested concentrations. The La and Ce contents, in all treatments, were significantly high in both shoots and roots ($p < 0.01$). Regarding La uptake levels, *Helianthus* accumulated around 3, 4, 3, and 8 µg g^{-1} FW in shoots and 8, 17, 40, and 76 µg g^{-1} FW in roots in 1, 2.5, 5, and 10 µM La concentrations, respectively. Similarly, an increase in Ce content was found ($p < 0.01$) in shoots (0.75, 1.5, 2, and 7 µg g^{-1} FW) and in roots (13, 22, 52, and 138 µg g^{-1} FW) following the Ce increase in the growth medium (from 1 to 10 µM).

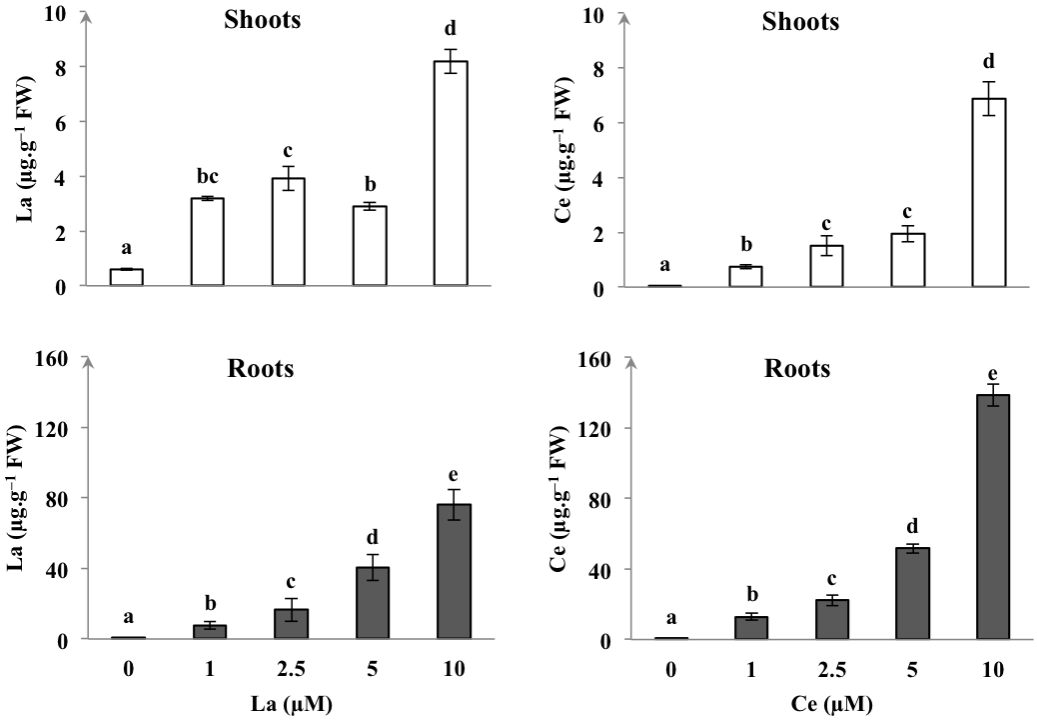

Figure 7. Lanthanum (La) and Cerium (Ce) contents in the shoots and roots of *Helianthus annuus* plants exposed to La (on the left side) or Ce (on the right side) for 14 days of treatment (values are means, n = 9). Values with different letters were significantly different at $p < 0.01$.

4. Discussion

4.1. REEs Influence on Plant Growth

According to obtained results described above (Figure 1), the shoot lengths increased only in 2.5 and 5 µM Ce-treated plants, whereas the root length was enhanced only at 10 µM La and 1 and 10 µM Ce. Several studies have proven that REE can stimulate plant growth and yield; La and Ce application positively affected the growth of maize plants [36], while Huang et al.'s study [37] revealed that the root growth of soybean was improved under the effect of La. Kovaříková et al.'s results [24] showed that an appropriate amount of REE could enhance plant growth due to their physiological effects' similarities with some mineral nutrients. However, a decline in root length at 2.5 µM Ce was noted, which could be explained by the decrease of cell division and the inhibition of mitotic activity at this concentration or it might be due to DNA damage, which may lead to the decrease of root growth rate [38,39].

4.2. Chlorophyll Fluorescence and Pigment Content

When exposed to La (5 and 10 µM) and Ce (2.5 and 10 µM), the biosynthesis of Chl a, Chl b, and carotenoids was improved in *Helianthus annuus* (Figure 4). Fv/Fm was enhanced in all REE treatments but was statistically different only in 10 µM La and 2.5 and 10 µM Ce concentrations, while Fv'/Fm' also increased in all applied La and Ce exposure concentrations, but no significant difference with the control was shown (Table 1) ($p > 0.05$). NPQ, ETR, and PAR rates were also ameliorated in *Helianthus annuus* under the effect of La and Ce. These results are in agreement with Wang et al. [7] who reported that the application of a low concentration of $LaCl_3$ on tobacco and broad bean seedlings increased chlorophyll content and photosynthetic rate. Ce^{3+} exposure also enhanced the photosynthetic rate and the biosynthesis of chlorophyll in *Spinacia oleracea* and both La^{3+} and Ce^{3+} significantly accelerated the photosynthesis in spinach [24,40]. Xie et al.'s results [25] revealed that the activity of PSII protein complex, as well as the velocity of photosynthesis electron transport,

can be improved by La effect, whereas this metal significantly increased the photochemical efficiency of chloroplast and the electron transport in tobacco plants.

It is known that the presence of REEs could increase PSII reactions and might promote chlorophyll synthesis in various treated plants through the stimulation of Rubisco activity [25,41]. In fact, Kovaříková et al. [24] stated that REEs increase chlorophylls a and b and carotenoid content, as well as the efficiency of PSII photochemistry and electron transfer rates (ETR) in plants. Moreover, elevated chlorophyll contents in leaf may represent a developed strategy by plants to increase CO_2 assimilation [42,43] and reduce the accumulated stress caused by REEs. Chlorophylls could also play an important ROS scavengers' role and be involved in the plants' defensive system [44], and carotenoids have the same protective aspect which allows these molecules to intervene in the maintenance of the chloroplast membrane's integrity [45].

In addition, the increase of the non-photochemical quenching (NPQ) in La- and Ce-treated plants, defined as a photoprotective process, indicated the enhancement of thermal dissipation and thus, the photo-damage at the pigment level could be avoided [46,47].

4.3. Accumulation of H_2O_2 and MDA

Among metallic stress biomarkers, H_2O_2 and MDA were used to assess the potential toxicity of La and Ce in *Helianthus annuus.* It is known that H_2O_2 has many essential roles in the metabolism of plants, but the production of this ROS can be enhanced by different environmental stresses [48]. In the present study, both La and Ce treatments stimulated the production of the free radical H_2O_2 in *H. annuus* leaves. Our findings are in accordance with previous studies where Ce stress induced the increase of H_2O_2 levels in *Lemna minor* species [49], while H_2O_2 was also found significantly enhanced in leaves of soybean seedlings in La treatment [50].

The estimation of potential oxidative cell damage was carried out by the determination of one of the end products of lipid peroxidation, the MDA [2,34]. In this study, 2.5 µM La and 10 µM Ce significantly increased the MDA content in *Helianthus annuus* leaves. These results are in accordance with Grosjean et al. [2] who mentioned that MDA content was significantly increased in leaves of *Pytolacca icosandra* and *Pytolacca clavigera* grown in 10 µM REE medium. Zhang et al. [51] reported an increase of MDA in *Elodea nuttallii* under the effect of La stress. Ippolito et al. [1] explained that La can enhance lipid peroxidation through the disturbance of the orderly assembly of the membrane making it more sensitive to oxidative stress.

4.4. Antioxidant Enzymes Response

Abiotic stresses, including metallic stress, are able to induce an excessive generation of ROS and therefore an imbalance between these free radicals and antioxidants that leads to irreversible plant cell damage [50,52,53]. *Helianthus annuus* plants that were irrigated with the nutritive solution supplemented with different concentrations of La and Ce showed that SOD activity was higher than the control (Figure 6). Several studies also demonstrated that SOD activity was found increased in *Eichhornia crassipes*, *Oryza sativa*, and *Mentha arvensis* treated with Pb [54–56].

POD and CAT are two major antioxidative peroxidase enzymes that control the peroxidative damage of cell walls by removing H_2O_2 [21]. Regarding leaves' POD activity in the studied plant, results showed a stimulation of this enzyme in all applied La and Ce concentrations (Figure 6). These findings are in agreement with Wang et al. [57], who showed an increase of POD activity in *Hydrilla verticillata* plants treated with La and Ce. Results also showed an increase in CAT activity under the effect of 1, 2.5, and 5 µM La, while a significant decrease in this enzyme activity was noted in the plants treated with 10 µM La. In contrast, Ce treatment showed an increase in CAT at 2.5, 5, and 10 µM (Figure 6). Similar results were reported in Liang and Wang [50], where CAT activity increased with the lowest concentrations of La and decreased with the highest one in soybean leaves. Ce also enhanced CAT activity in rice [58].

As reported by Saldaña-Sánchez et al. [45], Ce could promote the activity of antioxidant enzymes and suppress ROS accumulation. The increased activity of this enzyme is related to the intracellular level of H_2O_2 [53]. Changes of CAT and POD activities in La and Ce treatments were similar, and the increase of these enzymes may be due to the obtained high level of H_2O_2 in *Helianthus annuus* leaves. Moreover, the decrease in CAT activity in 10 µM La conversely to that of POD indicates that CAT was more sensitive than POD to the highest concentration of La in *Helianthus annuus* plant [50]. Kovaříková et al. [24] reported that SOD, CAT, and POD enzymes could be activated by varied REE concentrations that reached up to 200 µM; nonetheless, the antioxidative enzymatic response remains related to plant species and growth conditions.

4.5. REEs Accumulation

According to the obtained REEs accumulation status of *Helianthus annuus* in the present study (Figure 7), it was clearly proven that this plant can survive in the presence of La or Ce stress at the tested concentrations and during the chosen exposure period. Results also showed that the plants preferentially accumulated REEs in their roots. Ma et al. [59] also mentioned that most of the La and Ce was accumulated in the roots of cucumber plants. Moreover, from the findings described above, we notice that the accumulated La in shoots was higher than that of Ce, conversely to the roots, where Ce was remarkably higher than La. This could be explained by the fact that Ce can be more toxic for *Helianthus* plants than La. The difference in REE content in shoots and roots—$REE_{roots} > REE_{shoots}$—might be due a protection strategy adopted by the plants to reduce the transport of these metals from roots (through the apoplastic barriers) into the upper parts in order to avoid any toxic effect that may ruin the metabolic mechanisms and cause irreversible cell damage [36,60]. This behaviour makes *Helianthus annuus* one of numerous species that developed an adaptation against REE toxicity (including La and Ce) such as *Lemna gibba* and *Lemna minor* [61].

5. Conclusions

To summarise, neither La nor Ce treatments induced remarkably visible toxic signs in *Helianthus annuus* during the exposure period even with the highest REE concentrations. The REEs-treated plants did not show negative effects on the chlorophyll fluorescence including PSII photochemical efficiency, NPQ, ETR, and PAR, whereas these parameters were found increased under the effect of La and Ce. Moreover, chlorophylls and carotenoid contents increased in the highest applied REE concentrations. The increase in stress markers, such as H_2O_2 and MDA levels, without any marked deleterious effect on the plant can be explained by the effectiveness of the defence response through the stimulation of SOD, POD, and CAT which protects the plant from oxidative damage. In addition, this sunflower was found susceptible to safely absorbing and accumulating both La and Ce in its organs (for the most part in roots). The study of the response of this species to other REEs is suggested.

Author Contributions: Conceptualisation, N.S. and I.C.; methodology, N.D., P.B., H.B. and N.S.; formal analysis, N.D., H.B. and R.F.; writing—original draft preparation, N.D. and R.F.; writing—review and editing, P.B. and S.M.-D.; visualisation, S.M.-D. and I.C.; supervision, N.S. and I.C. All authors have read and agreed to the published version of the manuscript.

Funding: The authors received no funding for this article.

Institutional Review Board Statement: Not applicable. The manuscript does not contain any data on animal research or human research.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work was financially supported by LISBOA-01-0145-FEDER-031863 project, co-funded by FEDER through POR Lisboa (Programa Operacional Regional de Lisboa) from Portugal 2020 and Fundação para a Ciência e Tecnologia (PTDC/CTA-AMB/31863/2017). The authors also

gratefully acknowledge the support of CERENA (strategic project FCT-UIDB/04028/2020) and MARE (strategic project FCT-UIDB/04292/2020).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ippolito, M.P.; Fasciano, C.; d'Aquino, L.; Morgana, M.; Tommasi, F. Responses of antioxidant systems after exposition to rare earths and their role in chilling stress in commosn duckweed (*Lemna minor* L.): A Defensive Weapon or a Boomerang? *Arch. Environ. Contam. Toxicol.* **2010**, *58*, 42–52. [CrossRef] [PubMed]
2. Grosjean, N.; Jean, M.L.; Berthelot, C.; Chalot, M.; Gross, E.M.; Blaudez, D. Accumulation and fractionation of rare earth elements are conserved traits in the Phytolacca genus. *Sci. Rep.* **2019**, *9*, 18458. [CrossRef]
3. Elbasan, F.; Ozfidan-Konakci, C.; Yildiztugay, E.; Kucukoduk, M. Rare-earth element scandium improves stomatal regulation and enhances salt and drought stress tolerance by up-regulating antioxidant responses of *Oryza sativa*. *Plant Physiol. Biochem.* **2020**, *152*, 157–169. [CrossRef] [PubMed]
4. Tyler, G. Rare earth elements in soil and plant systems—A review. *Plant Soil* **2004**, *267*, 191–206. [CrossRef]
5. Balaram, V. Rare earth elements: A review of applications, occurrence, exploration, analysis, recycling, and environmental impact. *Geosci. Front.* **2019**, *10*, 1285–1303. [CrossRef]
6. Li, X.X.; Yu, B.; Dong, Y.Y.; Wang, L.S.; Zhang, S.L.; Shangguan, H.Y.; He, Z.H.; Luo, X.M.; Lai, P.F. Lanthanum chloride enhances the photosynthetic characteristics and increases konjac glucomannan contents in *Amorphophallus sinensis* Belval. *Photosynthetica* **2020**, *58*, 165–173. [CrossRef]
7. Wang, L.; Wang, W.; Zhou, Q.; Huang, X. Combined effects of lanthanum (III) chloride and acid rain on photosynthetic parameters in rice. *Chemosphere* **2014**, *112*, 355–361. [CrossRef] [PubMed]
8. Yang, Q.; Li, Y.; Wang, L.; Zhou, Q.; Huang, X. Effect of lanthanum (III) on the production of ethylene and reactive oxygen species in soybean seedlings exposed to the enhanced ultraviolet-B radiation. *Ecotoxicol. Environ. Saf.* **2014**, *104*, 152–159. [CrossRef]
9. Duarte, A.C.O.; De Oliveira, C.; Ramos, S.J.; De Castro, E.M.; Siqueira, J.O.; Guilherme, L.R.G. Lanthanum content and effects on growth, gas exchanges, and chlorophyll index in maize plants. *Acta Sci. Biol. Sci.* **2018**, *40*, e38469. [CrossRef]
10. Cao, Z.; Stowers, C.; Rossi, L.; Zhang, W.; Lombardini, L.; Ma, X. Physiological effects of cerium oxide nanoparticles on the photosynthesis and water use efficiency of soybean (*Glycine max* (L.) Merr.). *Environ. Sci. Nano J.* **2017**, *4*, 1086. [CrossRef]
11. Wang, L.; Li, J.; Zhou, Q.; Yang, G.; Ding, X.L.; Li, X.; Cai, C.X.; Zhang, Z.; Wei, H.Y.; Lu, T.H.; et al. Rare earth elements activate endocytosis in plant cells. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 12936–12941. [CrossRef] [PubMed]
12. Liu, D.; Wang, X.; Lin, Y.; Chen, Z.; Xu, H.; Wang, L. The effects of cerium on the growth and some antioxydant metabolisms in rice seedlings. *Environ. Sci. Pollut. Res.* **2012**, *19*, 3282–3291. [CrossRef] [PubMed]
13. Hu, H.; Wang, L.; Li, Y.; Sun, J.; Zhou, Q.; Huang, X. Insight into mechanism of lanthanum (III) induced damage to plant photosynthesis. *Ecotoxicol. Environ. Saf.* **2016**, *127*, 43–50. [CrossRef] [PubMed]
14. Bankaji, I.; Pérez-Clemente, R.M.; Caçador, I.; Sleimi, N. Accumulation potential of Atriplex halimus to zinc and lead combined with NaCl: Effects on physiological parameters and antioxidant enzymes activities. *S. Afr. J. Bot.* **2019**, *123*, 51–61. [CrossRef]
15. D'Aquino, L.; de-Pinto, M.C.; Nardi, L.; Morgana, M.; Tommasi, F. Effect of some light rare earth elements on seed germination, seedling growth and antioxidant metabolism in *Triticum durum*. *Chemosphere* **2009**, *75*, 900–905. [CrossRef] [PubMed]
16. Hu, X.; Ding, Z.; Chen, Y.; Wang, X.; Dai, L. Bioaccumulation of lanthanum and cerium and their effects on the growth of wheat (*Triticum aestivum* L.) seedlings. *Chemosphere* **2002**, *48*, 621–629. [CrossRef] [PubMed]
17. Hu, H.; Wang, L.; Zhou, Q.; Huang, X. Combined effects of simulated acid rain and lanthanum chloride on chloroplast structure and functional elements in rice. *Environ. Sci. Pollut. Res.* **2016**, *23*, 8902–8916. [CrossRef] [PubMed]
18. Dai, H.P.; Shan, C.J.; Wei, A.Z.; Yang, T.; Sa, W.Q.; Feng, B. Leaf senescence and photosynthesis in foxtail millet [*Setariaitalica* (L.) P. Beauv] varieties exposed to drought conditions. *Aust. J. Crop Sci.* **2012**, *6*, 232–237.
19. Bouslimi, H.; Ferreira, R.; Dridi, N.; Brito, P.; Martins-Dias, S.; Caçador, I.; Sleimi, N. Effects of Barium stress in *Brassica juncea* and *Cakile maritima*: The indicator role of some antioxidant enzymes and secondary metabolites. *Phyton* **2021**, *90*, 145. [CrossRef]
20. Bhaduri, A.M.; Fulekar, M.H. Antioxidant enzyme responses of plants to heavy metal stress: Review. *Rev. Environ. Sci. Biotechnol.* **2012**, *11*, 55–69. [CrossRef]
21. Berni, R.; Luyckx, M.; Xu, X.; Legay, S.; Sergeant, K.; Hausman, J.F.; Lutts, S.; Caia, G.; Guerrierod, G. Reactive oxygen species and heavy metal stress in plants: Impact on the cell wall and secondary metabolism. *Environ. Exp. Bot.* **2018**, *161*, 98–106. [CrossRef]
22. Sat, I.G. The effect of heavy metals on peroxidase from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. *Afr. J. Biotechnol.* **2008**, *7*, 2248–2253.
23. Nas, F.S.; Ali, M. The effect of lead on plants in terms of growing and biochemical parameters: A review. *MOJ Ecol. Environ. Sci.* **2018**, *3*, 265–268.
24. Kovaříková, M.; Tomášková, I.; Soudek, P. Rare earth elements in plants: Review. *Biol. Plant.* **2019**, *63*, 20–32. [CrossRef]
25. Xie, Y.; Cai, X.; Liu, W.; Tao, G.; Chen, Q.; Zhang, Q. Effects of lanthanum nitrate on growth and chlorophyll fluorescence characteristics of *Alternanthera philoxeroides* underperchlorate stress. *J. Rare Earths* **2013**, *31*, 823. [CrossRef]

26. Stirbet, A. Govindjee On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient: Short review. *J. Photochem. Photobiol. B Biol.* **2011**, *104*, 236–257. [CrossRef] [PubMed]
27. Ferreira, R.A.; Duarte, J.G.; Vergine, P.; Antunes, C.D.; Freire, F.; Martins-Dias, S. *Phragmites* sp. physiological changes in a constructed wetland treating an effluent contaminated with a diazo dye (DR81). *Environ. Sci. Pollut. Res.* **2014**, *21*, 9626–9643. [CrossRef]
28. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.* **1987**, *148*, 350–382.
29. Bouazizi, H.; Jouili, H.; Geitmann, A.; El-Ferjani, E. Copper toxicity in expanding leaves of *Phaseolus vulgaris* L.: Antioxidant enzyme response and nutrient element uptake. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 1304–1308. [CrossRef]
30. Velikova, V.; Loreto, F. On the relationship between isoprene emission and thermo-tolerance in *Phragmites australis* leaves exposed to high temperatures and during the recovery from a heat stress. *Plant Cell Environ.* **2005**, *28*, 318–327. [CrossRef]
31. Davis, D.G.; Swanson, H.R. Activity of stress-related enzymes in the perennial weed leafy spurge (*Euphorbia esula* L.). *Environ. Exp. Bot.* **2001**, *46*, 95–108. [CrossRef]
32. Brito, P.; Ferreira, R.A.; Martins-Dias, S.; Azevedo, O.M.; Caetano, M.; Caçador, I. Cerium uptake, translocation and toxicity in the salt marsh halophyte *Halimione portulacoides* (L.), Aellen. *Chemosphere* **2021**, *266*, 128973. [CrossRef]
33. Ferreira, R.A.; Martins-Dias, S. Purification of plant complex protein extracts in non-denaturing conditions by in-solution isoelectric focusing. *Anal. Biochem.* **2016**, *509*, 100–103. [CrossRef]
34. Labidi, O.; Vives-Peris, V.; Gómez-Cadenas, A.; Pérez-Clemente, R.M.; Sleimi, N. Assessing of growth, antioxidant enzymes, and phytohormone regulation in *Cucurbita pepo* under cadmium stress. *Food Sci. Nutr.* **2021**, *9*, 2021–2031. [CrossRef] [PubMed]
35. Sleimi, N.; Bankaji, I.; Kouki, R.; Dridi, N.; Duarte, B.; Caçador, I. Assessment of extraction methods of trace metallic elements in plants: Approval of a common method. *Sustainability* **2022**, *14*, 1428. [CrossRef]
36. Vilela, L.A.F.; Ramos, S.J.; Carneiro, M.A.C.; Faquin, V.; Guilherme, L.R.G.; Siqueira, J.O. Cerium (Ce) and Lanthanum (La) promoted plant growth and mycorrhizal colonization of maize in tropical soil. *Aust. J. Crop Sci.* **2018**, *12*, 704–710. [CrossRef]
37. Huang, G.R.; Wang, L.H.; Zhou, Q. Combined effects of Lanthanum (III) and elevated ultraviolet-B radiation on root growth and ion absorption in soybean seedlings. *Environ. Sci. Pollut. Res.* **2013**, *21*, 3621–3633. [CrossRef] [PubMed]
38. Kotelnikova, A.; Fastovets, I.; Rogova, O.; Volkov, D.S.; Stolbova, V. Toxicity assay of lanthanum and cerium in solutions and soil. *Ecotoxicol. Environ. Saf.* **2019**, *167*, 20–28. [CrossRef] [PubMed]
39. Wang, C.; Zhang, K.; He, M.; Jiang, C.; Tian, L.; Tian, Y.; Wang, X. Mineral nutrient imbalance, DNA lesion and DNA-protein crosslink involved in growth retardation of *Vicia faba* L. seedlings exposed to lanthanum ions. *J. Environ. Sci.* **2012**, *24*, 214–220. [CrossRef]
40. Wu, M.; Wang, P.Y.; Sun, L.G.; Zhang, J.J.; Yu, J.; Wang, Y.W.; Chen, G.X. Alleviation of cadmium toxicity by cerium in rice seedlings is related to improved photosynthesis, elevated antioxidant enzymes and decreased oxidative stress. *Plant Growth Regul.* **2014**, *74*, 251–260. [CrossRef]
41. Emmanuel, E.S.C.; Anandkumar, B.; Natesan, M.; Maruthamuthu, S. Efficacy of rare earth elements on the physiological and biochemical characteristics of *Zea mays* L. *Aust. J. Crop Sci.* **2010**, *4*, 289–294.
42. Sanchez-Zabala, J.; González-Murua, C.; Marino, D. Mild ammonium stress increases chlorophyll content in *Arabidopsis thaliana*. *Plant Signal. Behav.* **2015**, *10*, e991596. [CrossRef] [PubMed]
43. Caçador, I.; Duarte, B.; Marques, J.C.; Sleimi, N. Carbon mitigation: A salt marsh ecosystem service in times of change. In *Halophytes for Food Security in Dry Landss*; Khan, M.A., Ozturk, M., Gul, B., Ahmed, M.Z., Eds.; Elsevier Academic Press: Amsterdam, The Netherlands, 2016; pp. 83–110. [CrossRef]
44. Agathokleous, E.; Feng, Z.Z.; Peñuelas, J. Chlorophyll hormesis: Are chlorophylls major components of stress biology in higher plants? *Sci. Total Environ.* **2020**, *726*, 138637. [CrossRef]
45. Saldaña-Sánchez, W.D.; León-Morales, J.M.; López-Bibiano, Y.; Hernández-Hernández, M.; Langarica-Velázquez, E.C.; García-Morales, S. Effect of V, Se, and Ce on Growth, Photosynthetic Pigments, and Total Phenol Content of Tomato and Pepper Seedlings. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 678–688. [CrossRef]
46. Gao, Y.; Huang, W.; Zhu, L.; Chen, J. Effects of $LaCl_3$ on the growth and photosynthetic characteristics of Fny-infected tobacco seedlings. *J. Rare Earths* **2012**, *30*, 725. [CrossRef]
47. Lambrev, P.H.; Miloslavina, Y.; Jahns, P.; Holzwarth, A.R. On the relationship between non-photochemical quenching and photoprotection of Photosystem II. *Biochim. Biophys. Acta* **2012**, *1817*, 760–769. [CrossRef]
48. Cheeseman, J.M. Hydrogen Peroxide and Plant Stress: A Challenging Relationship. *Plant Stress* **2007**, *1*, 4–15.
49. Zicari, M.A.; d'Aquino, L.; Paradiso, A.; Mastrolitti, S.; Tommasi, F. Effect of cerium on growth and antioxidant metabolism of *Lemna minor* L. *Ecotoxicol. Environ. Saf.* **2018**, *163*, 536–543. [CrossRef] [PubMed]
50. Liang, C.; Wang, W. Antioxidant response of soybean seedlings to joint stress of lanthanum and acid rain. *Environ. Sci. Pollut. Res.* **2013**, *20*, 8182–8191. [CrossRef] [PubMed]
51. Zhang, J.; Zhang, T.; Lu, Q.; Cai, S.; Chu, W.; Qiu, H.; Xu, T.; Li, F.; Xu, Q. Oxidative effects, nutrients and metabolic changes in aquatic macrophyte, *Elodea nuttallii* following exposure to lanthanum. *Ecotoxicol. Environ. Saf.* **2015**, *115*, 159–165. [CrossRef] [PubMed]

52. Hasanuzzaman, M.; Hossain, M.A.; Teixeira-da-Silva, J.A.; Fujita, M. Plant Response and Tolerance to Abiotic Oxidative Stress: Antioxidant Defense Is a Key Factor. In *Crop Stress and Its Management: Perspectives and Strategies*; Springer: Dordrecht, The Netherlands, 2012; pp. 261–315. [CrossRef]
53. Sleimi, N.; Kouki, R.; Hadj-Ammar, M.; Ferreira, R.; Pérez-Clemente, R. Barium effect on germination, plant growth, and antioxidant enzymes in *Cucumis sativus* L. plants. *Food Sci. Nutr.* **2021**, *9*, 2086–2094. [CrossRef] [PubMed]
54. Malar, S.; Shivendra-Vikram, S.; Favas, P.J.C.; Perumal, V. Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. *Bot. Stud.* **2016**, *55*, 54. [CrossRef]
55. Thakur, S.; Singh, L.; Zularisam, A.W.; Sakinah, M.; Din, M.F.M. Lead induced oxidative stress and alteration in the activities of antioxidative enzymes in rice shoots. *Plant Biol.* **2017**, *61*, 595–598. [CrossRef]
56. Nigam, N.; Khare, P.; Yadav, V.; Mishra, D.; Jain, S.; Karak, T.; Panja, S.; Tandon, S. Biochar-mediated sequestration of Pb and Cd leads to enhanced productivity in *Mentha arvensis*. *Ecotoxicol. Environ. Saf.* **2019**, *172*, 411–422. [CrossRef]
57. Wang, X.; Shi, G.X.; Xu, Q.S.; Xu, B.J.; Zhao, J. Lanthanum and cerium induced oxidative stress in submerged *Hydrilla verticillata* plants. *Russ. J. Plant Physiol.* **2007**, *54*, 693–697. [CrossRef]
58. Xu, Q.M.; Chen, H. Antioxidant responses of rice seedling to Ce (4+) under hydroponic cultures. *Ecotoxicol. Environ. Saf.* **2011**, *74*, 1693–1699. [CrossRef] [PubMed]
59. Ma, Y.; Zhang, P.; Zhang, Z.; He, X.; Li, Y.; Zhang, J.; Zheng, L.; Chu, S.; Yang, K.; Zhao, Y.; et al. Origin of the different phytotoxicity and biotransformation of cerium and lanthanum oxide nanoparticles in cucumber. *Nanotoxicology* **2015**, *9*, 262–270. [CrossRef] [PubMed]
60. Rezaee, A.; Hale, B.; Santos, R.M.; Chiang, Y.W. Accumulation and Toxicity of Lanthanum and Neodymium in Horticultural Plants (*Brassica chinensis* L. and *Helianthus annuus* L.). *Can. J. Chem. Eng.* **2017**, *96*, 2263–2272. [CrossRef]
61. Sasmaz, M.; Obek, E.; Sasmaz, A. The accumulation of La, Ce and Y by *Lemna minor* and *Lemna gibba* in the Keban gallery water, Elazig Turkey. *Water Environ. J.* **2018**, *32*, 75–83. [CrossRef]