Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

Effect of cerium on growth and antioxidant metabolism of *Lemna minor* L.

Maria Alessandra Zicari[a], Luigi d'Aquino[b], Annalisa Paradiso[a], Silvio Mastrolitti[c], Franca Tommasi[a,*]

[a] *Department of Biology, University of Bari Aldo Moro, Via Orabona 4, 70125 Bari, Italy*
[b] *ENEA Portici Research Centre, Piazzale E. Fermi 1, 80055 Portici, Italy*
[c] *ENEA Territorial Office Bari, Viale Japigia 188, 70126 Bari, Italy*

ABSTRACT

An increasing input rate of rare earth elements in the environment is expected because of the intense extraction of such elements form their ores to face human technological needs. In this study *Lemna minor* L. plants were grown under laboratory conditions and treated with increasing concentrations of cerium (Ce) ions to investigate the effects on plant growth and antioxidant systems. The growth increased in plants treated with lower Ce concentrations and reduced in plants treated with higher concentrations, compared to control plants. In plants treated with higher Ce concentrations lower levels of chlorophyll and carotenoid and the appearance of chlorotic symptoms were also detected. Increased levels of hydrogen peroxide, antioxidant metabolites and antioxidant activity confirmed that higher Ce concentrations are toxic to *L. minor*. Ce concentration in plant tissues was also determined and detectable levels were found only in plants grown on Ce-supplemented media. The use of duckweed plants as a tool for biomonitoring of Ce in freshwater is discussed.

1. Introduction

Rare earth elements (REEs) include elements from lanthanum to lutetium, known as "lanthanides" plus scandium and yttrium (Evans, 1983; Ni, 1995). They share similar chemical properties and accumulate in the same ore deposits. Cerium (Ce) is one of the most abundant element among REEs, displaying a wide range of human applications. The utilization of REEs in a broad array of industrial processes took place in the recent decades, making REEs indispensable ingredients in many technological applications. In addition, REEs utilization in agriculture, in animal husbandry and also in medicine is well known (Tommasi and d'Aquino, 2017; d'Aquino and Tommasi, 2017). Chloride and nitrate forms of Ce and lanthanum (La) are the main constituent of REE micro-fertilisers that are used in China since the 1970s to improve crop yield (Hu et al., 2004). Following the dramatic increase of Ce-containing materials reaching the environment, such as wastes, by-products of REEs extraction, fertilizers, manure etc., biogeochemical cycles of REEs have been heavily altered and abnormal accumulation of such elements in the environment is expected to take place. An increasing amount of data about the biological effects of REEs, often controversial, are available from 1980's about effects on terrestrial plants. For example, Lopez-Moreno et al. (2010a) reported an increase in root growth in cucumber and corn, and a stimulation of reproductive growth and floral initiation was reported in *Arabidopsis thaliana* by He and Loh (2002, 2000). Chen et al. (2004) found that both Ce and La exert stimulatory effects on *Crocus sativus*, enhancing crocin production (Ce) and growth (La). Other authors reported that Ce treatments can alleviate the effects of mineral deficiencies (Chao et al., 2008; Hao et al., 2008; Zhou et al., 2011; Ze et al., 2009) and UV-related stress (Liang et al., 2011). Fashui (2002) reported that Ce treatments on aged *Oryza sativa* seeds contrasted seed aging, promoting seed germination and antioxidant enzyme activities. In addition, different authors reported that Ce enhanced photosynthesis (Fashui et al., 2002; Xiaoqing et al., 2009; Yuguan et al., 2009), mitochondrial activity (Dai et al., 2011) and nitrogen metabolism (Weiping et al., 2003). Ce treatments seem to promote transgene integration in some species (Boyko et al., 2011) and to preserve fruit degradation due to pesticide accumulation (Wu et al., 2010). On the other hand, adverse effects of Ce on plants

---

were observed by Diatloff et al. (2008) in mung bean and genotoxic effects were also reported in soybean by Lopez-Moreno et al. (2010b), that also found that application of Ce nanoparticles decreased seed germination in maize and root growth in alfalfa and tomato (Lopez-Moreno et al., 2010a). An increase in reactive species of oxygen (ROS) and inhibition of several antioxidants following Ce treatments was observed in rice (Xu and Chen, 2011) and impairment in macronutrient metabolism has been reported in horseradish (Guo et al., 2007). Increased ROS production and apoptosis induced by Ce were also reported in *Taxus tricuspidata* plants (Yang et al., 2009) and cells (Ge et al., 2006). Toxicity of REEs at micromolar concentration was reported for the alga *Skeletonema costatum* (Tai et al., 2010). Significant accumulation of REEs has been detected in different organisms and even in bacteria and lichens (Bayer and Bayer, 1991; Paoli et al., 2014). Even if REEs are today considered as new and emerging contaminants in the ecosystems (Pagano et al., 2015a, 2015b; Xu et al., 2017), little information about their concentration in aquatic environments is so far available (Kulaksız and Bau, 2011; Protano and Riccobono, 2002) and, consequently, no regulatory thresholds for REEs levels and emissions to the environment have been so far indicated. Few information is also available about the responses of aquatic plants to REEs treatment (Tommasi and d'Aquino, 2017). For example, Xu et al. (2017) recently reported toxic effects in *Spirodela polyrhiza* treated with Ce at micromolar concentrations.

The aim of this work was to clarify the potential toxicity of Ce in aquatic environment collecting information about Ce effects in common duckweed *Lemna minor* L.*,* an aquatic species widely studied for biomonitoring and bioremediation pollutants in freshwater (Basiglini et al., 2018; Forni and Tommasi, 2016). The effect of Ce supply on growth, pigment content, lipid peroxidation levels, ROS levels and antioxidant metabolism were investigated under laboratory conditions up to millimolar concentrations, considered as threshold levels for polluted water sites (Zhu et al., 2012), since lipid peroxidation, pigment alteration, ROS and antioxidant levels were proposed as toxicity markers (Ippolito et al., 2010). We also investigated the effect of pH on Ce plant responses and tested Ce uptake to determine if duckweed can be used for biomonitoring of Ce-polluted freshwater.

2. Materials and methods

2.1. Chemicals

Ce(III) nitrate, Ce(III) chloride and Ce(IV) sulphate solutions were prepared dissolving commercial reagents (analytical grade, Sigma Aldrich, 99% purity) in Knop nutrient solution (Knop, 1865) containing: 8.4 mM CaN_2O_6 $4H_2O$, 9.89 mM KNO_3, 8.31 mM $MgSO_4$ $7H_2O$, 7.35 mM H_2KO_4P and 0.36 mM $FeSO_4$ $7H_2O$. Solutions were filter sterilized and stored at room temperature prior to use.

2.2. Plant material and treatments

A strain of *L. minor,* registered at the *Herbarium Horti Barensis* (BI) n. 41980, was obtained from the collection of the Botanical Garden of the University Aldo Moro in Bari and botanical identification was confirmed on a phenotypic basis following Pignatti et al. (2017).

The plants were surface sterilized in 10–20% (v/v) bleach for 30 s, rinsed with sterile distilled water twice and then acclimatized in Knop nutrient solution in a growth chamber for 15 days. At the beginning of the acclimatization phase Cefotaxine 100 µg/ml was added to the culture media to prevent bacterial contamination. Knop nutrient solution was added every 3 days to maintain the solution level. Growing conditions were 24 ± 2 °C, 90 µmol m^{-2} s^{-1} white light and 16 h/8 h light/dark photoperiod. For the growth tests, plants with two fronds were transferred to culture multiwell plates (15 ml and 34 mm of diameter) each containing Ce nitrate at concentrations 0, 2.5, 5, 10, 20 µM and 0.1, 0.5, 1 mM in Knop's nutrient solution pH solution 5.5 or 4. The

growth was measured after 3, 5, 7, 12 and 15 days as described below. To test the effects of different Ce forms, treatments with Ce chloride, Ce sulphate 0.5 and 1 mM for 7 days at pH 5.5 were also carried out as described above. For other experiments, plants were incubated in Petri dishes with Knop solution (pH 5.5) supplemented with Ce nitrate at concentrations 0, 2.5, 5, 10, 20 µM and 0.1, 0.5, 1 mM and were harvested after 5, 7 and 12 days growing. Knop nutrient solution and Knops nutrient solution (as described above) supplemented with Ca nitrate concentrations were used as controls in order to exclude nitrate effects. All treatments were applied with five replicates.

2.3. Plant growth

The plant growth was determined as relative growth rate (RGR) a parameter as suggested by the Iso/Dis 20079 (2004) protocol and calculated as follows:

$$RGR = (\ln N_t - \ln N_0) / t$$

In which N_0 is the number of fronds at the beginning of the experiment, N_t is the number of fronds at the selected exposure time and t is the exposure time (0, 3, 5, 7, 12 and 15 days).

2.4. Chlorophyll and carotenoids content

Plant samples (0.2 g) were homogenized at 4 °C with 80% acetone 1:15 w/v and the homogenates were centrifuged at 14,000g for 15 min. The absorbance at λ 663.2, λ 646.8 and λ 470 nm was determined on the supernatant using a Beckmann DU-600 spectrophotometer and the pigment contents were determined as reported by Lichtenthaler (1987).

2.5. Hydrogen peroxide content

Plant samples (0.3 g) were homogenized in 5% TCA 1:6 w/v and the homogenates were centrifuged at 14,000g for 20 min. Hydrogen peroxide content was determined in the supernatant using POD type I as described by Konigshofer et al. (2008).

2.6. Lipid peroxidation levels

Plant samples (0.4 g) were homogenized with 0.1% TCA 1:6 w/v and the homogenates were centrifuged at 12,000g for 15 min. The supernatant (0.5 ml) was mixed with 20% TCA 1:1 v/v containing 0.5% TBA w/v. Lipid peroxidation was determined by measuring the MDA amount obtained by the reaction with TBA, as reported by Ippolito et al. (2010).

2.7. ASC and GSH content

Plant samples (0.4 g) were homogenized with 5% metaphosphoric acid 1:2 w/v at 4 °C and the homogenates were centrifuged at 20,000g for 15 min. Total ASC and GSH content was determined on the supernatant as described by Paradiso et al. (2008).

2.8. Total antioxidant activity

Plant samples (0.2 g) were homogenized with 85% ethanol 1:6 w/v and the homogenates were centrifuged at 20,000g for 15 min. The total antioxidant activity was determined as reported by Teow et al. (2007) using ABTS as radical reacting with the different antioxidant molecules. The ABTS absorbance at λ 730 nm was determined after 1 min reaction.

2.9. Enzymatic assays

Plant samples (0.5 g) were homogenized in 50 mM Tris-Cl pH 7.8 containing 0.3 mM mannitol, 1 mM EDTA, 0.05% cysteine and 10 mM magnesium chloride 1:2 w/v. The homogenates were centrifuged at

25,000*g* for 20 min and the supernatants were used to determine the total protein content as reported by Bradford (1976) using BSA (Sigma) as a standard. APX (EC 1.11.1.11), DHAR (EC 1.8.5.1), MDHA-R (EC 1.6.5.4) and GR (EC 1.6.4.2) activities were determined as described by Ippolito et al. (2010). CAT (EC 1.11.1.6) activity was determined by measuring the change in absorbance at λ 240 nm as described by Paciolla et al. (2008). POD (EC1.11.1.7) activity was determined by measuring TMB oxidation in the presence of hydrogen peroxide at λ 652 nm as described by Paradiso et al. (2016).

2.10. Ce uptake

The determination of Ce content was performed as reported by d'Aquino et al. (2009b) with little modifications. Briefly, the plant biomass was mineralized by microwave assisted acidic (65% ultrapure nitric acid) digestion and then diluted and analysed by inductive coupled quadrupole mass spectrometer (ICP-MS, Agilent 7700 ×) equipped with an octopole reaction system. External calibration was performed using Ce nitrate standard solutions in the concentration range 1 ÷ 10 ng/l. Rh (100 ng/l) was used as internal standard. Monitoring the mass 140 Ce (with 0,3 amu, AMU. resolution) was ever obtained a correlation coefficient higher than 0.999. Ce content in the tissues was expressed as mg/g of dry weight. The precision and accuracy of the analysis was previously tested spiking several *L. minor* samples collected from the same batch. It was chosen the spiking method because it is not commercially available the certified reference material (*L minor* contaminated by Ce). For every spiked sample the recovery was always almost quantitative (higher than 95%) and the relative mean absolute difference from the expected value never greater than 5%.

2.11. Statistical analyses

All results are presented as mean values ± standard deviation (SD). The experimental data were subjected to a two way analysis of variance (ANOVA). When the differences were significant (P-values < 0.05) Tukey's tests were performed for post hoc comparisons. Tests were performed with XLSTAT 2018 software.

3. Results

3.1. Plant growth

The appearance of chlorotic symptoms were observed after 5 days in plants treated with Ce 1 mM and after 12 days in plants treated with Ce 0.1 mM and 0.5 mM (Fig. 1).

No effects on RGR and on all tested parameters were recorded in plants treated with any Ca nitrate concentration compared to the control (data not shown), therefore Knop nutrient solution without Ce supplementation was used as a control. No significant variations in RGR were recorded in plants following treatments with Ce at micromolar concentrations (data not shown). An increase in RGR was observed in plants treated with Ce 0.1 and 0.5 mM at all incubation times (Fig. 2A). Plants treated with 1 mM Ce showed a decrease of RGR after 7 days of treatment. The effect of different Ce salts on plant growth were reported in Fig. 2B. Nitrate and chloride forms showed a similar effect, inducing an increase in RGR at 0.5 mM and an heavy decrease at 1 mM concentrations. Ce sulphate did not inhibit the plant growth and induced an increase of RGR at 1 mM concentration.

RGR was negatively affected by all Ce treatments after 7 and 15 days when Ce-containing solutions were at pH 4. (Table 1).

3.2. Chlorophyll and carotenoids content

Chlorophyll a and b content decreased in plants treated with Ce 0.5 mM and 1 mM after 5 and 7 days of treatment. In the plants treated

with Ce 0.1 mM a significant decrease was observed after 7 and after 12 days for chlorophyll a (Fig. 3A, B). Carotenoids level decreased in plants treated with Ce 0.5 and 1 mM after 5, 7 and 12 days of treatment and in plants treated with Ce 0.1 mM after 12 days of treatment (Fig. 3C).

3.3. Hydrogen peroxide content

The hydrogen peroxide content increased significantly following treatments with Ce 1 mM at all sampling times and with 0.5 mM Ce after 7 and 12 days of treatments (Fig. 4A).

3.4. Lipid peroxidation levels

An increase in MDA content was observed in plants treated with Ce 1 mM after all exposure times and after 7 days in plants treated with Ce 0.5 mM (Fig. 4B).

3.5. Antioxidant molecules content and enzyme activity assays

An increase in total GSH content (reduced GSH plus oxidized forms) occurred in all samples, independently from the exposition time, except for plants treated with Ce 0.1 mM after 5 days of treatment (Fig. 5A). On the contrary, ASC increased only after 5 days of treatment (Fig. 5B). A decrease was observed in other experimental time, becoming significant only in plants treated with Ce 1 mM after 12 days of treatment. An increase of total antioxidants was recorded after 5 days of treatment with Ce 0.5 and 1 mM and a significant decrease was recorded after 12 days of treatment with Ce 0.5 and 1 mM (Fig. 5C). The activity of enzymatic antioxidant removal systems for hydrogen peroxide, such as APX, POD and CAT is reported in Fig. 5: a moderate increase of APX activity occurred only after 5 days of treatment with Ce 1 mM (Fig. 5D). In all other cases differences were not significant compared to control, except for a decrease recorded in plants treated with Ce 1 mM after 12 days. Treatments with Ce 0.1 mM, 0.5 mM and 1 mM induced a significant increase in POD activity after 5 and 7 days at 1 mM, whereas other treatments did not cause any significant effect on POD levels (Fig. 5E). No significant effects on CAT activity levels were detected (Fig. 5F). Treatments with all tested Ce concentrations and at all sampling times induced no significant variations in MDHAR, DHAR and GR activities.

3.6. Ce uptake

Ce concentration in the plants treated with Ce 0, 0.1 mM, 0.5 mM and 1 mM for 5, 7 and 12 days and in control plants are reported in Fig. 6. Ce was not detectable in the control plants whereas its concentration in plant tissues increased following the increase in Ce supplement in the growing media. Ce uptake was saturated after 5 days because no variations in Ce content was observed in plants after longer treatments.

4. Discussion

Our results indicate that *L. minor* tolerates Ce at millimolar concentrations and that Ce ions significantly affect metabolic processes in *L. minor*. In fact, treatments with Ce 0.5 mM increased the plant growth whereas Ce 1 mM inhibited plant growth. Our results agree only partially with those obtained by Xu et al. (2017) on a different duckweed species, *S. polyrhiza*, in which growth inhibition occurred already at micromolar Ce concentrations, thus suggesting that different duckweeds can display a different sensitivity to Ce ions in the growing media.

In our work treatments with Ce at pH 4 inhibited plant growth after 7 and 15 days at all tested concentrations while treatments at pH 5.5 inhibited the growth only with Ce 1 mM from 7 days of incubation. Furthermore, growth inhibition at pH 4 is accompanied by an increase
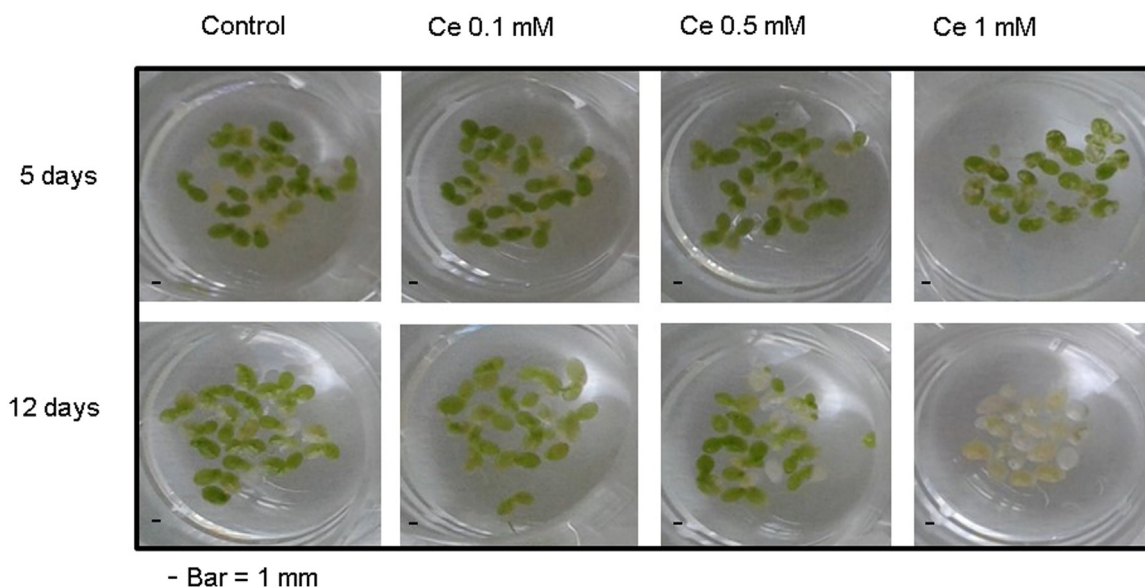
Fig. 1. *L. minor* plants treated with Ce nitrate at concentrations 0 (control), 0.1, 0.5 and 1 mM for 5 and 12 days.

in lipid peroxidation levels at all tested times and concentrations (data not shown), thus indicating that pH value modulates the plant-Ce interaction. This is in accordance with data in the literature that indicate that REEs toxicity is influenced by medium acidification (Wang et al., 2014) and that Ce accumulation, mobility and toxicity are greater at low pH values (Thomas et al., 2014).

In our experimental conditions, Ce chloride and nitrate forms induced similar effects on *Lemna* plants, both inducing a growth decrease at 1 mM concentration, while Ce sulphate form did not significantly affect RGR. Our results indicate that, as expected, Ce effects on plants are influenced by its chemical form, reasonably because of different bioavailability of Ce in different chemical forms.

The effects of Ce on the growth of *L. minor* follow a dose-depending response with a stimulation at 0.1 and 0.5 mM concentrations and an inhibition at higher concentrations. This biphasic effect resembles the hormetic effect (Nascarella and Calabrese, 2017) and is consistent with data reported for *Arabidopsis thaliana* (Wang et al., 2012b) and rice (Liu et al., 2012) treated with Ce, but also for sea urchins treated with different REEs (Oral et al., 2010).

Growth inhibition was also associated to chlorotic symptoms and to alteration of metabolic stress markers. The decrease in pigment content could be related either to the disturbance in biosynthetic pathways and to an enhanced degradation associated to damages to chloroplast ultrastructure (Fu et al., 2014; Wang et al., 2007; Xu et al., 2012), and possibly also to Mg-replacement by Ce in the chlorophyll molecule

(Hong et al., 2002; Zhou et al., 2011). The decrease of chlorophylls and carotenoids can explain the appearance of chlorotic symptoms and contribute to explain effects of Ce on plant growth. Controversial data concerning effects of REEs on chlorophyll levels are reported in the literature: for instance, in rice seedlings, chlorophyll content increased at low Ce concentrations, but it decreased when Ce was added at 1 mM concentrations (Liu et al., 2012), whereas Ce and other REEs enhanced the efficiency of light absorption in *A. thaliana* (Xiaoqing et al., 2009). However, growth inhibition associated to chlorophyll alterations, in fact, have already been observed in *L. minor* (Ippolito et al., 2010) and in other aquatic plants treated with REEs, such as *Nymphoides peltata* (Fu et al., 2014), *Hydrilla verticillata* (Wang et al., 2007), *Hydrocharis dubia* (Xu et al., 2012).

Many data so far reported in the literature indicate that under abiotic stress conditions, that are associated to an over-production of ROS into cells, plant resistance depends on the ability of the plant to modulate antioxidant responses (Mittler, 2002). Our data confirm that Ce affects the ROS/antioxidant balance and this is in accordance with already reported data concerning the occurrence of oxidative stress caused by La and/or Ce (Babula et al., 2015; Liu et al., 2012; Wang et al., 2012a, 2007; Zhang et al., 2015). In our work, Ce treatments induced a dose-dependent increase of cellular hydrogen peroxide associated to enhanced levels in lipid peroxidation, that can be considered as typical symptom of cellular damage. The increase in ROS levels is correlated with an enhancement of antioxidant systems, particularly in
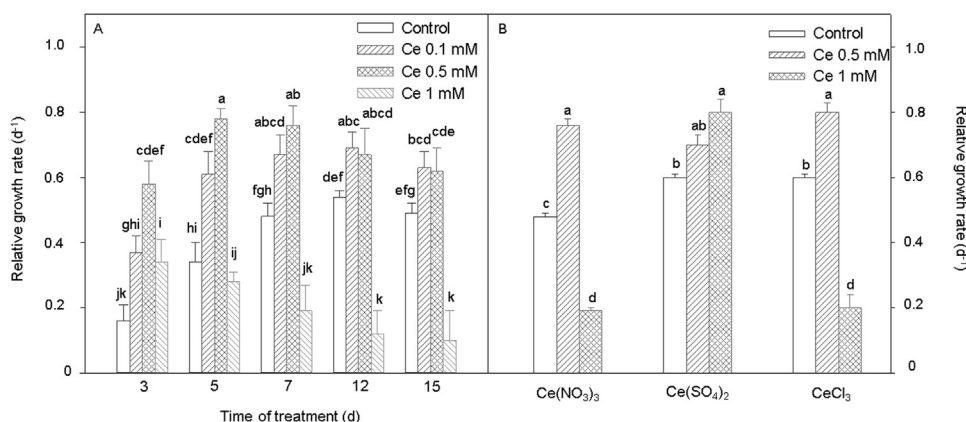

Fig. 2. Relative growth rate (RGR) in *L. minor* plants treated with Ce nitrate at concentrations 0 (control), 0.1, 0.5 and 1 mM for 15 days (A). RGR in *L. minor* plants treated for 7 days with Ce nitrate, Ce sulphate and Ce chloride at 0.5 and 1 mM concentrations (B). Values represent the mean ± SD of five experiments. Different letters indicate values that are statistically different.

Table 1

RGR, expressed as percentage of increment/decrease respect to the control in plants treated with 0.1, 0.5 and 1 mM Ce at pH 5.5 or 4. Measurement were performed between 0 and 3 d, 0 and 7, 0 and 15d. Control values of RGR are: 0.16 ± 0.05 after 3 days (d), 0.48 ± 0.04.after 7 d, and 0.49 ± 0.03 after 15 d at pH 5.5; 0.2 ± 0.05, after 3d, 0.4 ± 0.07, after 7 d, and 0.35 ± 0.05, after 15 d at pH 4. Values represent the mean (± SD) of five experiments. *values that are statistically different.

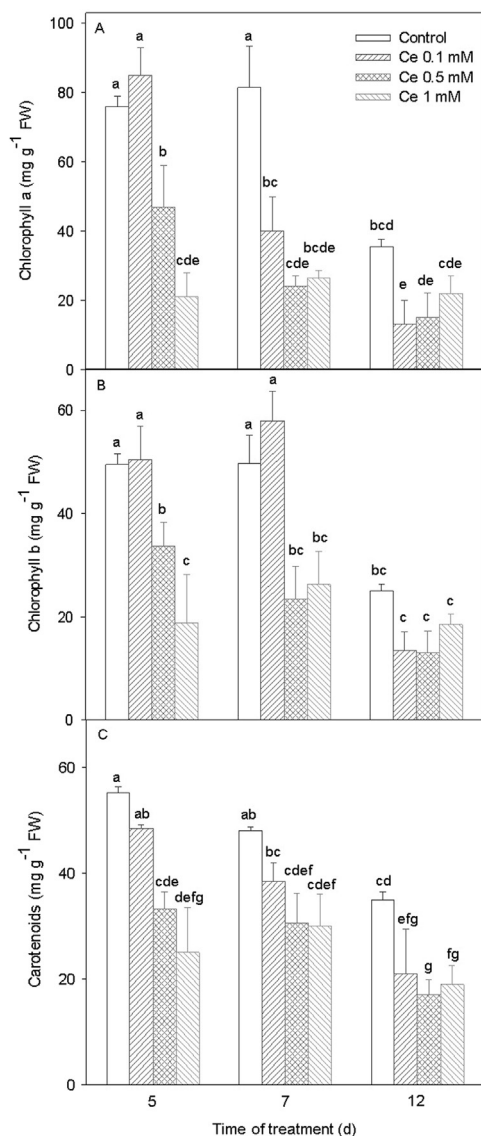| Treatment	RGR (%)					
	pH 5.5			pH 4		
	Ce 0.1 mM	Ce 0.5 mM	Ce 1 mM	Ce 0.1 mM	Ce 0.5 mM	Ce 1 mM
3 days	+ 130 ± 31 *	+ 260 ± 44 *	+ 110 ± 44 *	− 20 ± 30	− 60 ± 20	− 75 ± 40
7 days	+ 39 ± 12 *	+ 58 ± 12 *	− 60 ± 17 *	− 83 ± 15 *	− 88 ± 5 *	− 85 ± 10 *
15 days	+ 28 ± 10 *	+ 26 ± 14	− 80 ± 18 *	− 92 ± 6 *	− 92 ± 9 *	− 92 ± 12 *

Fig. 3. Chlorophyll a (A), chlorophyll b (B) and carotenoids (C) content in *L. minor* plants treated with Ce nitrate at concentrations 0 (control), 0.1, 0.5 and 1 mM for 5, 7 and 12 days. Values represent the mean ± SD of five experiments. Different letters indicate values that are statistically different.

Fig. 4. Hydrogen peroxide (A) and MDA (B) content in *L. minor* plants treated with Ce nitrate at concentrations 0 (control), 0.1, 0.5 and 1 mM for 5, 7 and 12 days. Values represent the mean ± SD of five experiments. Different letters indicate values that are statistically different.

the first days of treatment. In fact, plants exposed to Ce showed a significant increase of GSH and also an increase of ASC after 5 days of treatments, as reported in the response of plants to abiotic stresses (Apel and Hirt, 2004; Mishra et al., 2006; Seth et al., 2008) and also after REE treatments (d'Aquino et al., 2009a; Ippolito et al., 2010). The GSH level
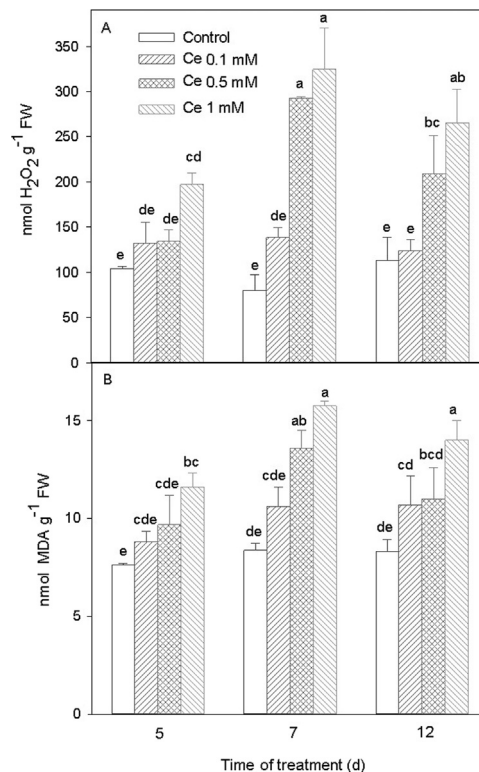
could be involved in the responses to REEs as well as in responses to heavy metals, in which the tripeptide induces specific proteins responsible of the detoxification processes (Paradiso et al., 2008; Xiang and Oliver, 1998). Data here reported suggest that Ce affects the antioxidant molecules more than antioxidant enzymes because only moderate alterations of APX and POD activities occurred and only after 5 days, and catalase activity was not significantly affected. Instead, the enzymes that control hydrogen peroxide balance in the plant cells are involved in response to Ce and this is in accordance with previously reported data (Liu et al., 2012; Wang et al., 2012b). The observed alterations of ROS production, lipid peroxidation and photosynthetic pigments levels are typical markers of Ce toxicity, as reported in the responses to abiotic stress (Radic et al., 2010; Wang et al., 2014; Xu et al., 2010; Zhang et al., 2015) and as suggested by Ippolito et al. (2010) for La and REEs mixtures. Ippolito et al. (2010) reported toxic effects of La and REEs mixture on *L. minor* only following supply of concentrations 5 and 10 fold higher than those used in this work, thus suggesting that Ce is more toxic for *L. minor* than La and other REEs.
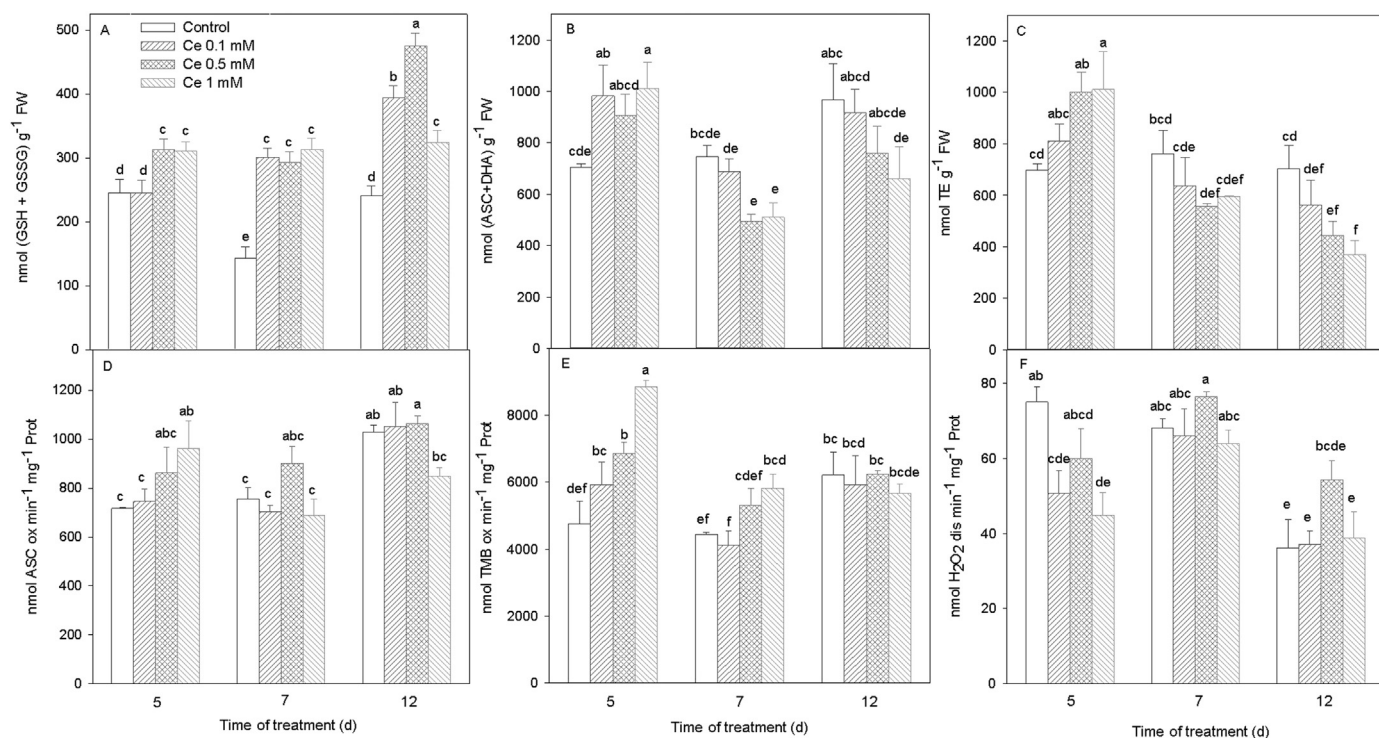
Fig. 5. Glutathione (A), Ascorbate (B) and Total Antioxidant content (C), APX (D), POD (E) and CAT (F) activities in *L. minor* plants treated with Ce nitrate at concentrations 0 (control), 0.1, 0.5 and 1 mM for 5, 7 and 12 days. Values represent the mean ± SD of five experiments. Different letters indicate values that are statistically different.
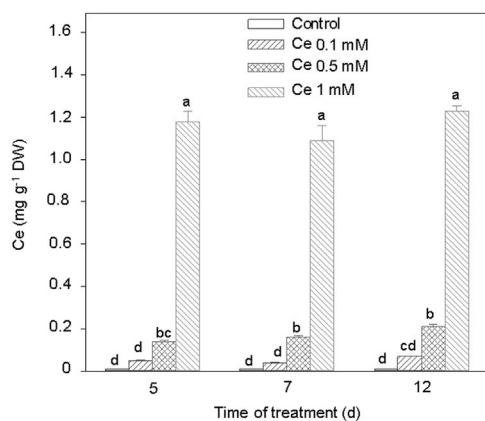

Fig. 6. Ce concentration in *L. minor* plants treated with Ce nitrate at concentrations 0 (control), 0.1, 0.5 and 1 mM for 5, 7 and 12 days. Values represent the mean ± SD of five experiments. Different letters indicate values that are statistically different.

Our results indicate that Ce is not naturally present in the tissues of *L. minor* at detectable amounts and it increased in plant tissues following addition of Ce in the growing media in a dose-dependent manner until saturation happens after 5 days of treatment at 1 mM concentration. Thus, effects of Ce supply on *L. minor* are related to its ability to enter in cells and to be accumulated in plant tissues. Further studies are needed to understand Ce cellular localization Ce accumulation in *L. minor* plants suggests a potential use of this species in biomonitoring of Ce-polluted aquatic environments, as already proposed for other aquatic plant species such as *S. polyrhiza* (Yang et al., 1999; Xu et al., 2017), *Eichhornia crassipes* (Chua, 1998), *Hydrocharisdubia* (Xu et al., 2012), *Nymphoides peltata* (Fu et al., 2014).

5. Conclusions

Ce ions affect growth and metabolism in *L. minor* following a biphasic trend, with stimulatory effects at lower concentrations and inhibitory effects at higher concentrations. Greater Ce concentrations also induced toxicity symptoms on *Lemna* fronds, but alteration of photosynthetic pigment content occurred even in absence of any visible effect. Alteration of ROS production, lipid peroxidation as well as the unbalance of antioxidant systems, i.e. typical markers for stress conditions, occurred after treatments with higher Ce concentrations, thus confirming the potential biological risk associated to Ce-accumulation in soil and freshwater. The tolerance of *L. minor* to low Ce levels in the environment and its ability to uptake this element suggests that this plant species is potentially a useful tool for biomonitoring of Ce-polluted freshwater.

Acknowledgements

References

Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373–399.

Babula, P., Kkejdus, B., Kovacik, J., Hedbavny, J., Hlavna, M., 2015. Lanthanum rather than cadmium induces oxidative stress and metabolite changes in *Hypericum perforatum*. J. Hazard Mater. 286, 334–342.

Basiglini, E., Pintore, M., Forni, C., 2018. Effects of treated industrial wastewaters 1 and temperatures2 on growth and enzymatic activities of duckweed (*Lemna minor* L.). Ecotoxicol. Environ. Safe.

Bayer, M.E., Bayer, M.H., 1991. Lanthanide accumulation in periplasmic space of Escherichia coli. B. J. Bacteriol. 173, 141–149.

Boyko, A., Matsuoka, A., Kovalchuk, I., 2011. Potassium chloride and rare earth elements improve plant growth and increase the frequency of the *Agrobacterium tumefaciens* mediated plant transformation. Plant Cell Rep. 30, 505–518.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal. Biochem. 72,

248–254.

Chao, L., Bofu, P., Weiqian, C., Yun, L., Hao, H., Liang, C., Xiaoqing, L., Xiao, W., Fashui, H., 2008. Influences of calcium deficiency and cerium on growth of spinach plants. Biol. Trace Elem. Res. 121, 266–275.

Chen, S., Zhao, B., Wang, X., Yuan, X., Wang, Y., 2004. Promotion of the growth of *Crocus sativus* cells and the production of crocin by rare earth elements. Biotechnol. Lett. 26, 27–30.

Chua, H., 1998. Bio-accumulation of environmental residues of rare earth elements in aquatic flora *Eichhornia crassipes* (Mart.) Solms in Guangdong Province of China. Sci. Total Environ. 214, 79–85.

d'Aquino, L., De Pinto, M.C., Nardi, L., Morgana, M., Tommasi, F., 2009a. Effect of some light rare earth elements on seed germination, seedling growth and antioxidant metabolism in *Triticum durum*. Chemosphere 75, 900–905.

d'Aquino, L., Morgana, M., Carboni, M.A., Staiano, M., Antisari, M.V., Re, M., 2009b. Effect of elements on the growth and lanthanide accumulation in different *Trichoderma strains*. Soil Biol. Biochem. 41, 2406–2413.

d'Aquino, L., Tommasi, F., 2017. Rare earth elements and microorganisms. In: Pagano, G. (Ed.), Rare Earth Elements in Human and Environmental Health: At the Crossroad Between Toxicity and Safety. Pan Stanford Publishing Pte. Ltd., pp. 127–141.

Dai, J., Liu, J.J., Zhou, G.Q., Zhang, Y.Z., Liu, Y., 2011. Effect of Ce(III) on heat pro-duction of mitochondria isolated from hybrid rice. Biol. Trace Elem. Res. 143, 1142–1148.

Diatloff, E., Smith, F.W., Asher, C.J., 2008. Effects of lanthanum and cerium on the growth and mineral nutrition of corn and mung bean. Ann. Bot. 101 (7), 971–982.

Evans, C.H., 1983. Interesting and useful biochemical properties of lanthanides. Trends Biochem. Sci. 8 (12), 445–449.

Fashui, H., 2002. Study on the mechanism of cerium nitrate effects on germination of aged rice seed. Biol. Trace Elem. Res. 87, 191–200.

Fashui, H., Ling, W., Xiangxuan, M., Zheng, W., Guiwen, Z., 2002. The effect of cerium (III) on the chlorophyll formation in spinach. Biol. Trace Elem. Res. 263–276.

Forni, C., Tommasi, F., 2016. Duckweed: a Tool for Ecotoxicology and a Candidate for Phytoremediation. Curr. Biotechnol. 5, 2–10.

Fu, Y.Y., Li, F.F., Xu, T., Cai, S.J., Chu, W.Y., Qiu, H., Sha, S., Cheng, G.Y., Xu, Q.S., 2014. Bioaccumulation, subcellular, and molecular localization and damage to physiology and ultrastructure in *Nymphoides peltata* (Gmel.) O. Kuntze exposed to yttrium. Environ. Sci. Pollut. Res. 21, 2935–2942.

Ge, Z., Q., Yang, S., Yuan, Y.J., 2006. Ce^{4+} induced down-regulation of ERK-like MAPK and activation of nucleases during the apoptosis of cultured *Taxus cuspidata* cells. J. Inorg. Biochem. 100, 167–177.

Guo, X., Zhou, Q., Lu, T., Fang, M., Huang, X., 2007. Distribution and translocation of 141 Ce(III) in horseradish. Ann. Bot. 100, 1459–1465.

Hao, H., Ling, C., Xiaoqing, L., Chao, L., Weiqian, C., Yun, L., Fashui, H., 2008. Absorption and transfer of light and photoreduction activities of spinach chloroplasts under calcium deficiency: promotion by cerium. Biol. Trace Elem. Res. 122, 157–167.

He, Y.W., Loh, C.S., 2002. Induction of early bolting in *Arabidopsis thaliana* by tria-contanol, cerium and lanthanum iscorrelated with increased endogenous con-centration of isopentenyl adenosine (iPAdos). J. Exp. Bot. 53 (368), 505–512.

He, Y.W., Loh, C.S., 2000. Cerium and lanthanum promote floral initiation and re-productive growth of *Arabidopsis thaliana*. Plant Sci. 159, 117–124.

Hong, F.S., Wang, L., Meng, X.X., 2002. The effect of Cerium (III) on the chlorophyll formation in spinach. Biol. Trace Elem. Res. 89 (46), 263–276.

Hu, Z., Richter, H., Sparovek, G., Schnug, E., 2004. Physiological and biochemical effect of rare earth elements on plants and their agricultural significance: a review. J. Plant Nutr. 27, 183–220.

Ippolito, M.P., Fasciano, C., d'Aquino, L., Morgana, M., Tommasi, F., 2010. Responses of antioxidant systems after exposition to rare earths and their role in chilling stress in common duckweed (*Lemna minor* L.): a defensive weapon or a boomerang? Arch. Environ. Contam. Toxicol. 58, 42–52.

Iso/Dis 20079, 2004. Water quality – determination of the toxic effect of water con-stituents and waste water to duckweed (*Lemna minor*) – Duckweed growth inhibition test. ISO TC 147/SC 5/WG 5.

Knop, W., 1865. Quantitative Untersuchungenüber die ErnahrungsprozessederPflanzen. Landwirtsch. Vers. Stn. 7, 93–107.

Konigshofer, H., Tromballa, H.W., Lopper, H.G., 2008. Early events in signalling high temperature stress in tobacco BY2 cells involve alterations in membrane fluidity and enhanced hydrogen peroxide production. Plant Cell Environ. 31, 1771–1780.

Kulaksız, S., Bau, M., 2011. Rare earth elements in the Rhine River, Germany: first case of anthropogenic lanthanum as a dissolved microcontaminant in the hydrosphere. Environ. Int. 37, 973–979.

Liang, C., Zhang, G., Zhou, Q., 2011. Effect of cerium on photosynthetic pigments and photochemical reaction activity in soybean seedling under ultraviolet-b radiation stress. Biol. Trace Elem. Res. 142, 796–806.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids – pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–382.

Liu, D., Wang, X., Lin, Y., Chen, Z., Xu, H., Wang, L., 2012. The effects of cerium on the growth and some antioxidant metabolisms in rice seedlings. Environ. Sci. Pollut. Res. 19, 3282–3291.

Lopez-Moreno, M.L., la Rosa, De, HernandezViezcas, G., Peralta Videa, J., Guardea Torresdey, J, J., 2010a. X-ray Absorption Spectroscopy (XAS) corroboration of the uptake and storage of CeO_2 nanoparticles and assessment of their differential toxicity in four edible plant species. J. Agric. Food. 58 (6), 3689–3693.

Lopez-Moreno, M.L., De la Rosa, G., HernandezViezcas, J., Castillo-Michel, H., Botez, C.E., Peralta-Videa, J.R., GardeaTorresdey, J.L., 2010b. Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (*Glycine max*) plants. Environ. Sci. Technol. 44 (19), 7315–7320.

Mishra, S., Srivastava, S., Tripathia, R.D., Govindarajan, R., Kuriakose, S.V., Prasad,

M.N.V., 2006. phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. Plant Physiol. Biochem. 44, 25–37.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410.

Nascarella, M.A., Calabrese, E.J., 2017. Hazard assessment and evaluation of Rare earth elements dose responses relationships. In: Pagano, G. (Ed.), Rare Earth Elements in Human and Environmental Health: At the Crossroad Between Toxicity and Safety. Pan Stanford Publishing Pte. Ltd., pp. 183–194.

Ni, J.Z., 1995. Bioinorganic chemistry of Rare Earth Elements. Science Press, Beijing, pp. 13–37.

Oral, R., Bustamante, P., Warnau, M., D'Ambra, A., Guida, M., Pagano, G., 2010. Cytogenetic and developmental toxicity of cerium and lanthanum to sea urchin embryos. Chemosphere 81, 194–198.

Paciolla, C., Ippolito, M.P., Logrieco, A., Mule`, A., Dipierro, N., Dipierro, S., 2008. A different trend of antioxidant defence responses makes tomato plants less susceptible to beauvericin than to T-2mycotoxin phytotoxicity. Physiol. Mol. Plant Pathol. 72, 3–9.

Pagano, G., Aliberti, F., Guida, M., Oral, R., Siciliano, A., Trifuoggi, M., Tommasi, F., 2015a. Rare earth elements in human and animal health: state of art and research priorities. Environ. Ecotoxicol. Saf. 142, 215–220.

Pagano, G., Guida, M., Tommasi, F., Oral, R., 2015b. Health effects and toxicity me-chanism of rare earth elements- Knowledge gaps and research prospects. Environ. Ecotoxicol. Saf. 115, 40–48.

Paoli, L., Fiorini, E., Munzi, S., Sorbo, S., Basile, A., Loppi, S., 2014. Uptake and acute toxicity of cerium in the lichen *Xanthoria parietina*. Ecotoxicol. Environ. Saf. 104, 379–385.

Paradiso, A., Berardino, R., de Pinto, M.C., Sanita` di Toppi, L., Storell, M.M., Tommasi, F., De Gara, L., 2008. Increase in ascorbate–glutathione metabolism as local and precocious systemic responses induced by cadmium in durum wheat plants. Plant Cell Physiol. 49, 362–374.

Paradiso, A., Caretto, S., Leone, A., Bove, A., Nisi, R., De, Gara L., 2016. ROS production and scavenging under anoxia and re-oxygenation in *Arabidopsis* cells: a balance be-tween redox-signalling and impairment. Front. Plant Sci. 7, 1803. https://doi.org/10.3389/fpls.2016.01803.

Pignatti, S., La Rosa, M., Guarino, R., 2017. Flora d'Italia. 1. Edagricole New Business Media, Bologna Italy, pp. 117.

Protano, G., Riccobono, F., 2002. High contents of rare earth elements (REE) in stream waters of a Cu-Pb-Zn mining area. Environ. Pollut. 117, 499–514.

Radic, S., Stipanicev, D., Cvjetko, P., Mikelic, I.L., Rajcic, M.M., Sirac, S., Pevalek-Kozlina, B., Pavlica, M., 2010. Ecotoxitological assessment of industrial effluent using duck-weed (*Lemna minor* L.) as a test organism. Ecotoxicology 19, 216–222.

Seth, C.S., Chaturvedi, P.K., Misra, V., 2008. The role of phytochelatins and antioxidants in tolerance to Cd accumulation in *Brassica juncea* L. Ecotoxicol. Environ. Saf. 71, 76–85.

Tai, P.D., Zhao, Q., Su, D., Li, P.J., Stagnitti, F., 2010. Biological toxicity of lanthanide elements on algae. Chemosphere 80, 1031–1035.

Teow, C.C., Truong, V.D., McFeeters, R.F., 2007. Antioxidant activities, phenolic and betacarotene contents of sweet potato genotypes with varying flesh colours. Plant Physiol. 126, 1214–1223.

Thomas, P.J., Carpenter, D., Boutin, C., Allison, J.E., 2014. Rare earth elements (REEs): effects on germination and growth of selected crop and native plant species. Chemosphere 96, 57–66.

Tommasi, F., d'Aquino, L., 2017. Rare earth elements and plants. In: Pagano, G. (Ed.), Rare Earth Elements in Human and Environmental Health: At the Crossroad Between Toxicity and Safety. Pan Stanford Publishing Pte. Ltd., pp. 107–125.

Wang, L.H., Wang, W., Zhou, Q., Huang, X.H., 2014. Combined effects of lanthanum (III) chloride and acid rain on photosynthetic parameters in rice. Chemosphere 112, 355–361.

Wang, X., Li, J., Lu, Y., Jin, H., Deng, S., Zeng, Y., 2012a. Effects of cerium on growth and physiological characteristics of *Anabaena flos-aquae*. J. Rare Earths 30, 1287–1292.

Wang, X., Lin, Y., Liu, D., Xu, H., Liu, T., Zhao, F., 2012b. Cerium toxicity, up take and translocation in *Arabidopsis thaliana* seedlings. J. Rare Earths 30, 579–585.

Wang, X., Shi, G.X., Xu, Q.S., Xu, B.J., Zhao, J., 2007. Lanthanum and Cerium Induced Oxidative Stress in Submerged *Hydrilla verticillata* Plants. Russ. J. Plant Physiol. 54 (5), 693–697.

Weiping, S., Fashui, H., Zhigang, W., Yuzhen, Z., Fugen, G., Hongoing, X., Mingliang, Y., Youhong, C., Mizhen, Z., Jiale, S., 2003. Effects of cerium on nitrogen metabolism of peach plantlet in vitro. Biol. Trace Elem. Res. 95, 259–268.

Wu, H., Wang, G.D., Shi, J., Xue, S., Gao, M., 2010. Effect of the complex of zinc(II) and cerium(IV) with chitosan on the preservation quality and degradation of organo-phosphorus pesticides in Chinese Jujube (*Zizyphus jujube* Mill. cv. Dongzao). J. Agric. Food Chem. 58, 5757–5762.

Xiang, C., Oliver, D.J., 1998. Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in Arabidopsis. Plant Cell. 10, 1539–1550.

Xiaoqing, L., Hao, H., Chao, L., Min, Z., Fashui, H., 2009. Physico-chemical Property Of Rare Earths-effects On The Energy Regulation Of Photosystem II in *Arabidopsis thaliana*. Biol. Trace Elem. Res. 130, 141–151.

Xu, Q., Jiang, Y., Chu, W., Su, C., Hu, D., Lu, Q., Zhang, T., 2017. Response of *Spirodela polyrhiza* to cerium: subcellular distribution, growth and biochemical changes. Ecotoxicol. Environ. Safe. 139, 56–64.

Xu, Q.M., Chen, H., 2011. Antioxidant responses of rice seedling to Ce^{4+} under hydro-ponic cultures. Ecotoxicol. Environ. Safe 74, 1693–1699.

Xu, Q.S., Fu, Y.Y., Min, H.L., Cai, S.J., Sha, S., Cheng, G.Y., 2012. Laboratory assessment of uptake and toxicity of lanthanum (La) in the leaves of *Hydrocharis dubia* (Bl.) Backer. Environ. Sci. Pollut. Res 19, 3950–3958.

Xu, Q.S., Hu, J.Z., Xie, K.B., Yang, H.Y., Du, K.H., Shi, G.X., 2010. Accumulation and

acute toxicity of silver in *Potamogeton crispus* L. J. Hazard Mater. 173, 186–193.

Yang, S., Lu, S.H., Yuan, Y.J., 2009. Cerium elicitor-induced phosphatidic acid triggers apoptotic signalling development in *Taxus cuspidata* cell suspension cultures. Chem. Phys. Lipids 159, 13–20.

Yang, X.Y., Ying, D.Q., Sun, H., Wang, X.R., Dai, L.M., Chen, J., Cao, M., 1999. Distribution and bioavailability of rare earth elements in aquatic microcosm. Chemosphere 39, 2442–2450.

Yuguan, Z., Min, Z., Luyang, L., Zhe, J., Chao, L., Sitao, Y., Yanmei, D., Na, L., Fashu, H., 2009. Effects of cerium on key enzymes of carbon assimilation of spinach under magnesium deficiency. Biol. Trace Elem. Res. 131, 154–164.

Ze, Y., Yin, S., Ji, Z., Luo, L., Liu, C., Hong, F., 2009. Influences of magnesium deficiency and cerium on antioxidant system of spinach chloroplasts. Biometals 22, 941–949.

Zhang, J., Zhang, T., Lu, Q., Cai, S., Chu, W., Qiu, H., Xu, T., Li, F., Xu, Q., 2015. Oxidative effects, nutrients and metabolic changes in aquatic macrophyte, *Elodea nuttallii*, following exposure to lanthanum. Ecotoxicol. Environ. Safe. 115, 159–165.

Zhou, M., Gong, X., Wang, Y., Liu, C., Hong, M., Wang, L., Hong, F., 2011. Improvement of cerium of photosynthesis functions of maize under magnesium deficiency. Biol. Trace Elem. Res. 142 (760-722).

Zhu, Z., Wang, Z., Li, J., Li, Y., Zhang, Z., Zhang, P., 2012. Distribution of rare earth elements in sewage-irrigated soil profiles in Tianjin, China. J. Rare Earths 30, 609–613.