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Oxidative effects, nutrients and metabolic changes in aquatic macrophyte, *Elodea nuttallii*, following exposure to lanthanum

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ABSTRACT

We investigated the phytoremediation potential of *Elodea nuttallii* to remove rare earth metals from contaminated water. The laboratory experiments were designed to assess the responses induced by lanthanum (5–20 mg L^{-1}) in *E. nuttallii* over a period of 7 days. The results showed that most La (approximately 85%) was associated with the cell wall. The addition of La to the culture medium reduced the concentration of K, Ca, Cu, Mg, and Mn. However, $O_2^{\cdot -}$ levels increased with a concomitant increase in the malondialdehyde (MDA) concentration as the La concentration increased, which indicated that the cells were under oxidative stress. Significant reductions in the levels of chlorophyll (Chl) *a*, *b*, and carotenoids (Car) were observed in a concentration-dependent manner. However, the levels of reduced glutathione (GSH), total non-protein thiols (TNP-SH) and phytochelatins (PCs) increased for all La concentrations. The results suggested that La was toxic to *E. nuttallii* because it induced oxidative stress and disturbed mineral uptake. However, *E. nuttallii* was able to combat La induced damage via an immobilization mechanism, which involved the cell wall and the activation of non-enzymatic antioxidant.

1. Introduction

Lanthanum (La) is a member of the rare earth elements (REEs), which comprise the lanthanides or lanthanoids from lanthanum to lutetium plus yttrium and scandium. They share similar physicochemical properties, but are generally different from the common transitional metals. In recent years, due to high-tech and medical applications and the intense use of fertilizers in agriculture and forestry, large amounts of REEs have been emitted to the soil and water environment (Elbaz-Poulichet et al., 2002; Oral et al., 2010). REEs are believed to have limited toxicity and are not significantly hazardous to the environment. However, their slow accumulation in the environment could become problematic (Thomas et al., 2014). Toxicological investigations have suggested that REE bioaccumulation might have significant adverse effects on aquatic biota (Barry and Meehan, 2000; Oral et al., 2010).

To date, the effect of REE accumulation on plants remains fragmentary and sometimes inconsistent. For example, La significantly promoted the reproductive growth of *Arabidopsis thaliana* (He and Loh, 2000) and *Nicotinana tabacum* (Sun et al., 2003), but in a hydroponic experiment, the addition of La decreased the growth of *Zea mays* and *Vigna radiate* (Diatloff et al., 2008) and inhibited primary root elongation in *Triticum aestivum* seedlings (Hu et al., 2002). Liu and Hasenstein (2005) reported that low La^{3+} concentrations (<1 μM) promoted *Z. mays* root growth while higher concentrations (>100 μM) inhibited the growth of this organ. It is likely that the La influences on the cell are complex and multiple, such as ion signaling and homeostasis effects (Liu and Hasenstein, 2005) or binding to aromatic rings, carboxyl groups and phospholipids (Abe and Takeda, 1988). These contradictory results may depend of plants species, growth stage and the concentration and type of REEs (He and Loh, 2000; Hu et al., 2002; Diatloff et al., 2008; d'Aquino et al., 2009; Ippolito et al., 2010; Thomas et al., 2014). Although the availability and toxicity of exogenous REEs to plants has attracted more attention recently (Chua, 1998; Yang et al., 1999; Weltje et al., 2002; Xu et al., 2012; Fu et al., 2014), The La phytoremediation potential of *Elodea nuttallii*, a widespread macrophyte species, has not been investigated to any great extent.

Aquatic plants are represented by a variety of algal and macrophytic species that occur in many types of habitats and they are often used in water quality monitoring and assessment (Lewis, 1995; Mohan and Hosetti, 1999). It has also been shown that aquatic macrophytes can uptake metals directly from water and accumulate them in their shoots because they are completely

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submerged and have a very thin cuticle (St-Cyr et al., 1994; Wang et al., 2010b, Regier et al., 2013). *E. nuttallii*, a submerged plant native to North America, is known for its fast growth rate and is easily cultured in the laboratory. It is also able to accumulate pollutants. Therefore, it has been widely used as model experimental system for bioaccumulation and ecotoxicological investigations (Barrat-Segretain, 2004; Thiébaut et al., 2010; Xing et al., 2010; Regier et al., 2013; Larras et al., 2013).

This study investigated the responses of *E. nuttallii* to exogenous La by measuring (i) the accumulation and sub-cellular distribution of La; (ii) changes in nutrient uptake; (iii) La-induced oxidative stress through the measurement of $O_2^{\cdot -}$, MDA changes and chlorophyll concentrations; and (iv) variations in non-enzymatic antioxidants (reduced GSH, TNP-SH and PCs). Overall these accumulation and distribution data should improve our understanding of the main cellular and molecular targets involved in the accumulation/distribution of La and further the potential use of *E. nuttallii* in the phytoremediation programs of aquatic bodies polluted with REEs.

2. Materials and methods

2.1. Plant material and growth conditions

Cleaned, healthy, 10 cm-long vegetative shoots of *E. nuttallii*, collected from Lake Pipa, Nanjing, China. All the materials were cultivated in 1/10 Hoagland solution under controlled conditions (114 μmol m^{-2} s^{-1} light irradiance, 14 h photoperiod and 25 °C/20 °C day/night). Then lanthanum was added to the containers in the form of $LaCl_3{\cdot}7H_2O$ in appropriated quantities to give the following treatments: 5, 10, 15, 20 mg L^{-1} [La^{3+}]. The tested concentrations of La to perform our survey were chosen within the range of previous toxicological investigations on plants (Liu and Hasenstein, 2005; d'Aquino et al., 2009; Ippolito et al., 2010; Thomas et al., 2014). Each treatment had three replicates, and all of the solutions were refreshed every 2 days during the 7-day culture period.

2.2. Tissue fraction and la analysis

After 7 days of metal exposure, the *Elodea* plants were harvested and washed with double distilled water and 20 mM EDTA to remove metals absorbed on the surface of the plant material. The sub-cellular distribution of La within the shoots was determined according to the method described by Xiong et al. (2009). Concentrations of La in cell wall, organelles and soluble fraction were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, Leeman labs, Prodigy, USA) after digestion with HNO_3 and $HClO_4$ (10:1, v/v) at 160°C (detection limit: 0.0015 mg/L). The liquid standard La reference material (GSB 04-1774-2004) was diluted and analyzed for La concentration.

2.3. Determination of mineral concentration

The mineral concentration in the shoots of *E. nuttallii* was determined according to previously reported methods (Xu et al., 2012; Fu et al., 2014). For each sample, 0.5 g of shoots was digested with HNO_3:$HClO_4$ (10:1, v/v) at 160 °C. The solution samples were diluted to a final volume of 10 mL and were analyzed for mineral elements (K, Ca, Fe, Cu, Zn, Mn, and Mg) by ICP-AES. Standard solution (xccc-13A, xccc-14A, SPEX CertiPrep, USA) were used for the calibration.

2.4. Determination of $O_2^{\cdot -}$ generation rate

The concentration of $O_2^{\cdot -}$ generation rate was measured using the hydroxylamine chloride method (Wang and Luo, 1990). The supernatant (0.5 mL) was collected and incubated at 25 °C for 60 min in the presence of 1 mM hydroxylamine hydrochloride in 50 mM sodium phosphate buffer (pH 7.8). The reaction mixture was then incubated with 1 mL of 17 mM P-aminobenzene sulfonic acid anhydrous and 1 mL of 7 mM a-naphthylamine at 25 °C for 30 min. The absorbance was measured at 530 nm. A calibration curve was established using sodium nitrite.

2.5. Lipid peroxidation

The level of lipid peroxidation in the shoots (0.5 g) was determined immediately by thiobarbituric acid reaction according to Heath and Packer (1968). The concentration of MDA was calculated using 155 mM cm^{-1} as extinction coefficient in terms of nmol g^{-1} fresh weight.

2.6. Determination of photosynthetic pigment concentration

Chlorophylls (Chl) and carotenoids (Car) were extracted with 80% acetone and absorbances at 470, 647 and 663 nm recorded on a spectrophotometer (Thermo GENESYS 10). The concentrations of Chl *a*, Chl *b* and Car were estimated according to Lichtenthaler (1987).

2.7. Reduced glutathione (GSH), total non-protein thiol (TNP-SH), and phytochelatins (PCs) determination

To determine the concentration of reduced GSH, fresh shoots (0.5 g) were homogenized in ice-cold 5% (w/v) trichloroacetic acid and then centrifuged at 10,000*g* for 20 min at 4 °C. The reduced GSH concentration was determined spectrophotometrically at 412 nm according to the method used by Anderson (1985), after precipitation with 0.1 M HCl, using GSH reductase, 5,5′–dithio–bis-(2-nitrobenzoic acid) (DTNB) and NADPH. The reduced GSH concentration was expressed as mg g^{-1} fresh weight.

In order to estimate the total non-protein thiol (TNP-SH) concentration, fresh shoots were extracted in 6.67% 5-sulphosalicylic acid and centrifuged at 12,000*g* for 10 min at 4 °C. Supernatant was reacted with Ellman's reagent (5 mM EDTA and 0.6 mM 5, 5′ dithiobis (2-nitroenzoic acid) in 120 mM phosphate buffer, pH 7.5) and absorbance was measured spectrophotometrically after 5 min at 412 nm (Ellman, 1959).

The PCs were measured by following the method of Bhargava et al. (2005). The PCs were measured using the formula: total NP-SH–GSH after the total NP-SH and reduced GSH concentrations had been determined.

2.8. Statistical analysis

All results are presented as mean values ± standard deviation (SD) of at least three independent experiments. Statistical analysis was performed by computer software SPSS17.0. A correlation analysis was performed to determine the relationships between La accumulation and studies parameters. The experimental data was subjected to a one-way analysis of variance (ANOVA) and *P*-values < 0.05 were considered significant.