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Molecular distribution and toxicity assessment of praseodymium by *Spirodela polyrrhiza*

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HIGHLIGHTS

- Pr was mainly bound to cellulose and pectin in *Spirodela polyrrhiza*.
- Pr induced the change in C—O, C—H, and O—H stretching vibration by FTIR.
- Pr induced per oxidation of unsaturated fatty acids in membrane lipids.
- Pr could target the reaction center proteins D1 and D2.
- The reduction in photo system II activity was detected under Pr exposure.

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ABSTRACT

Aquatic macrophytes are known to accumulate and bioconcentrate metals. In this study, the physiological, biochemical, and ultrastructural responses of *Spirodela polyrrhiza* to elevated concentrations of praseodymium (Pr), ranging from 0 to 60 μM, were investigated over 20 d exposure. The results showed that the accumulation of Pr in *S. polyrrhiza* occurred in a concentration-dependent manner. The accumulation of Pr in biomacromolecules decreased in the order of cellulose and pectin (65–69%), crude proteins (18–25%), crude polysaccharides (6–10%), crude lipids (3%–4%). Significant increases in malondialdehyde (MDA), and decreases in photosynthetic pigment, soluble protein, and unsaturated fatty acids showed that Pr induced oxidative stress. Inhibitory effects on photosystem II and the degradation of the reaction center proteins D1 and D2 were revealed by chlorophyll *a* fluorescence transients, immunoblotting, and damage to chloroplast ultrastructure. Significant increases in cell death were observed in Pr-treated plants. However, *S. polyrrhiza* can combat Pr induced oxidative injury by activating various enzymatic and non-enzymatic antioxidants. These results will improve understanding of the biological consequences of rare earth elements (REEs) contamination, particularly in aquatic bodies.

1. Introduction

Rare earth elements (REEs) include scandium (Sc), yttrium (Y), and lanthanide series (La-Lu), all of which possess similar chemical and physical properties. China has about 80 percent of the world's total REEs [1]. REEs have been widely applied in agriculture because of their positive effects on crop yield and quality [2]. The germinating rate of winter wheat, scallion, onion, and eggplants was increased after blending with appropriate REEs concentration [3]. Moreover, the increase use of REEs in ceramic manufacture, glass production, metallurgy, energy, and medicine leads to a large number of releases and over-accumulation of REEs in the environment, and then enters plants and aquatic systems [4–7]. The REEs bioaccumulation in the plants can transfer through the food chain to the human body, affecting the food safety and the human health [8]. It has been predicted that industrial and agricultural usage of REEs and the resulted environmental contamination will grow quickly in the next few decades [9]. Hence, the accumulation of REEs in the environmental has been attracting more and more attention.

REEs can be taken up through the leaf surface and roots of plants, and they show dose-dependent accumulation in plants [10]. Information about the influence of REEs on plant development remains contradictory [11]. It has been reported that the addition of Ce (0.5–50 μM) or La (0.5–50 μM) increased *Arabidopsis thaliana* root length [12]. Suitable concentrations of REEs could enhance the chlorophyll content, improve the photosynthetic rate, and increase the absorption of mineral nutrients [13–15]. However, many experiments have investigated the inhibitory effect of REEs on plants

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Table 1
Pr content in different biological molecules (crude lipids, crude polysaccharides, crude proteins, and cellulose and pectin) of *S. polyrrhiza* leaves. Data are means ± S.D. (n = 3). Different superscript letters indicate significantly values at $p < 0.05$.

Pr content (μg g^{-1} DW)	Pr concentration (μM)			
	0	20	40	60
Crude lipids	N.D.	206.40^{c} ± 16.70 (4)	314.22^{b} ± 5.08 (4)	382.87^{a} ± 3.86 (3)
Crude polysaccharides	N.D.	286.60^{c} ± 78.36 (6)	610.73^{b} ± 16.91 (8)	1199.14^{a} ± 70.44 (10)
Crude proteins	N.D.	1307.46^{c} ± 94.70 (25)	1904.61^{b} ± 6.59 (23)	2211.49^{a} ± 120.65 (18)
Cellulose and pectin	N.D.	3329.40^{c} ± 88.92 (65)	5331.56^{b} ± 248.88 (65)	8337.79^{a} ± 349.48 (69)
Total	N.D.	5129.86^{c} ± 67.44 (100)	8161.12^{b} ± 267.30 (100)	12131.29^{a} ± 295.41 (100)

[16–18]. For example, 1 mM La reduced primary root elongation and caused maize root tips to swell [19]. Around 50–500 μM Ce could decrease growth and photosynthesis, and destroy the chloroplast ultrastructure of *A. thaliana* seedlings [20]. REEs can also cause significant oxidative stress, nutrient imbalance, and cell damage [21–23]. Pr is a light REEs that is widely available in the environment and in REE fertilizer [24,25]. Tang and Tong [26] showed that low concentration of Pr could increase the radical growth in Chinese cabbage, but that high concentration of Pr prevented root growth. However, as far as can be ascertained, very little information is available on the accumulation and toxicity of Pr in aquatic plants.

In this study, *Spirodela polyrrhiza*, a floating plant, was used as an experimental model to investigate REEs induced response because of its fast growth, wide distribution, short life span, and sensitivity to various pollutants [27–31]. Researchers have found that *S. polyrrhiza* could be a good option for As, Cd phytoremediation and was very useful for biomonitoring of Ni contaminated waste water for the high phytotoxic sensitivity against of Ni [29–31]. Hence, *S. polyrrhiza* became a model plant to study the bioaccumulation and ecotoxicology of Pr. The objective of this investigation was to gain insight into the Pr toxicity mechanism in *S. polyrrhiza* from the following aspects: (1) bioaccumulation of Pr; (2) distribution of Pr in biological molecules; (3) the changes to antioxidant enzymes and antioxidants; (4) the composition of membrane fatty acids; (5) the ultrastructure of chloroplasts; (6) photochemical activity; and (7) through Fourier transform infrared spectroscopy (FTIR) analysis.

2. Materials and methods

2.1. Plant material and experimental design

S. polyrrhiza was collected from Nanjing, China and cultivated under laboratory conditions (114 μmol m^{-2} s^{-1} light irradiance, 14 h photoperiod and 25 °C/18 °C day/night temperature) in 1/10 Hoagland solution for at least one week. Similar fronds were then transferred to glass beakers. The plants were grown in 1/10Hoagland solution, but KH_2PO_4 was not added to avoid REEs precipitation [21,23,32]. Pr was added at 0, 20, 40, or 60 μM to the glass beakers in the form of $PrCl_3$ under the above mentioned laboratory conditions for 20 days. All solutions were renewed every 4 days and 0.1 mM KH_2PO_4 was applied to the foliage at the end of each photoperiod [32]. All treatments were carried out in triplicate.

2.2. Leaf damage analysis

To obtain the proportion of leaf area damaged (PLAD), an Epson Perfection V700 Photo (J221A, Japan) and WinFolia PRO 2011 software (Regent Instruments Inc., Canada) were used according to Fu et al. [23].

2.3. Chemical form extraction

The gradual extraction method was adopted according to Lai et al. [33] with slight modification: (1) 0.2 g dry *S. polyrrhiza* leaves were ground to a powder and extracted with ether for 8 h (Soxhlet extraction) at 45 °C to obtain the crude lipids; (2) the residue obtained from step 1 was extracted with 15 mL boiling water for 3.5 h and centrifuged at 800 × *g* for 10 min (repeated twice). Then the supernatants were combined to obtain the crude polysaccharides; (3) the resulting residue obtained from step 2 was extracted with 10 mL 0.1 M NaOH at 80 °C for 2 h, and then centrifuged at 10,000 × *g* for 10 min (repeated twice). The resulting supernatant contained the crude proteins, and the residue contained cellulose and pectin. Pr contents in the above four components were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Leeman Labs, USA) after decomposition by HNO_3 and $HClO_4$.

2.4. FTIR analysis

FTIR analysis was carried out according to Üçüncü et al. [34] with modifications. The leaves were ground into fine powder in liquid nitrogen. The FTIR experiments were carried out with a Nicolet model NEXUS 670 FTIR spectrometer (Nicolet Corporation, USA). The spectrometer was purged with CO_2-free dry air for 24 h before recording the spectra. The FTIR spectra were recorded between 400 cm^{-1} and 4000 cm^{-1} at room temperature (25 °C) using a resolution of 0.09 cm^{-1}. Each spectrum was automatically normalized to obtain the relative absorbance.

2.5. Chloroplast ultrastructure

Fresh leaves from the control and Pr-treated plants were initially fixed with 4% glutaraldehyde and then post-fixed with 1% osmic acid. After dehydration through a gradient ethanol series,

Table 2
Effect of Pr on photosynthetic pigment (Chl a, Chl b, and Car) and chlorophyll a fluorescence parameters (F_o, F_m, and F_v/F_m). Data are means ± S.D. (n = 3). Different superscript letters indicate significantly values at $p < 0.05$.

Parameters	Pr concentration (μM)			
	0	20	40	60
Chl a (mg g^{-1} FW)	0.36^{a} ± 0.04	0.20^{b} ± 0.01	0.19^{b,c} ± 0.01	0.16^{c} ± 0.01
Chl b (mg g^{-1} FW)	0.17^{a} ± 0.02	0.15^{b} ± 0.00	0.12^{b,c} ± 0.01	0.11^{c} ± 0.02
Car (mg g^{-1} FW)	0.07^{a} ± 0.00	0.06^{b} ± 0.00	0.06^{b} ± 0.00	0.05^{b} ± 0.01
F_o	385.67^{a} ± 9.07	333.33^{b} ± 24.42	280.33^{c} ± 17.01	278.00^{c} ± 8.00
F_m	2328.67^{a} ± 89.23	1951.67^{b} ± 7.37	1717.67^{b} ± 361.19	1594.00^{b} ± 179.12
F_v/F_m	0.83^{a} ± 0.00	0.81^{b} ± 0.01	0.79^{c} ± 0.01	0.63^{d} ± 0.02