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Lead sequestration in iron plaques developed on *Phalaris arundinacea* Linn. and *Carex cinerascens* Kukenth. from Poyang Lake (China)



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ABSTRACT

Iron plaque often forms on the roots of wetland plants under anaerobic conditions, and it may play a role in the uptake and translocation of metals in wetland plants. A hydroponic experiment was conducted to investigate whether iron plaque could affect the uptake and translocation of lead by two dominant wetland plants, *Carex cinerascens* Kukenth. and *Phalaris arundinacea* Linn., growing in Poyang Lake, Jiangxi Province, China. After iron plaque on plant roots was induced by growing plants in solution containing 0, 30, 60, 100, 150, or 210 mg L⁻¹ Fe²⁺ for 7 days, plants were transferred to nutrient solution containing 10 mg L⁻¹ Pb²⁺ for 5 days. A significant positive correlation was observed between the content of iron plaque and Fe²⁺ concentration in the solution for both the species. The content of iron plaque formed on the root surfaces of *C. cinerascens* with higher root activity was slightly more than that of *P. arundinacea* with lower root activity. Iron plaque on the root surfaces of the two species could adsorb or co-precipitate with Pb and could immobilize Pb on the root surfaces. The Pb concentrations and percentage distribution in different organs were in the order iron plaque > root > shoot. The findings that the proportions of Pb in DCB extracts and roots (nearly 90% in the present study) were significantly higher than that in shoots had been observed. The results showed that iron plaque on the root surfaces could increase sequestration of Pb in the root and reduce the translocation Pb from root to shoot under hydroponic conditions.

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1. Introduction

The iron plaque are formed due to the release of oxygen and oxidants in the rhizosphere and the subsequent oxidation of ferrous to ferric iron and the precipitation of iron oxides and hydroxides on the root surfaces (Armstrong 1964, 1967; Chen et al., 1980; Taylor et al., 1984). Many wetland plants can form iron plaques on the root surface when submerged in water, such as Oryza sativa L. (Liu et al., 2006, 2011; Ma et al., 2013), Lobelia dortmanna L. (Christensen and Wigand, 1998; Holmer et al., 1998), Typha latifolia L. (Ye et al., 1998; Zhong et al., 2010), Phragmites communis Trin. (Batty et al., 2002), Salix (Zimmer et al., 2011), mangrove (Pi et al., 2011). Chemically, iron plaque is a mixture of crystalline and amorphous iron oxides and hydroxides (Hansel et al., 2001; Liu et al., 2006), which is an amphoteric colloid with a high adsorption capacity and numerous environmental and ecological effects. For example, iron plaques may sequester a number of metal(loid)s by adsorption or co-precip itation, and thus interfere with the availability of these elements in the rhizosphere and their uptake by plants. Therefore, the presence of an iron plaque on root surfaces may influence metal(loid)s uptake (Otte et al., 1989; Liu et al., 2007).

To date no consensus has been reached on the interaction between iron plaques on roots and heavy metal uptake by the plants. Whereas several authors suggested that iron plaque can act as a "barrier" to the uptake of heavy metals, such as Cu (Ye et al., 1997; Batty et al., 2000), Pb (Liu et al., 2011), Ni (Greipsson 1995; Ye et al., 1997), and As (Garnier et al., 2010; Liu et al., 2006; Lee et al., 2013). Iron plaque may act as a "buffer" for Sb (V) and Sb (III) in the rhizosphere (Huang et al., 2011; Okkenhaug et al., 2012). Iron plaque can also sequester Al and Se on the root surface and reduce translocation of Al from roots to shoots (Zhou and Shi, 2007; Cai et al., 2012). Others (Liu et al., 2007, 2011) have observed the opposite, namely that an iron plaque on the root surface has little effect on uptake and translocation of Cd by rice plants. Furthermore, Zhang et al. (1998) reported that the effect of iron plaque on the uptake of toxic metals and nutrient elements may be related to the quantitative development of the iron plaque.

Carex cinerascens Kukenth. and Phalaris arundinacea Linn. are dominant species in Poyang Lake wetland; their cover area accounts for 60% of the total vegetation cover area (Zhang et al.,

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2012, 2013). These plants play an important role in purifying water quality, ameliorating eutrophication, removing metals (Gong et al., 2006). Based on the previous studies, Poyang Lake wetland has been polluted by metals Pb, Cd, Cu, Zn (concentrations of Pb, Cd, Cu, Zn were 56–125 mg kg⁻¹, 0.05–5 mg kg⁻¹, 23–254 mg kg⁻¹,120–368 mg kg⁻¹ respectively (Gong et al., 2006). Moreover, the Fe concentration in Poyang Lake wetland was generally higher than the national average content of soil (Ren, 2012). *C. cinerascens* and *P. arundinacea* were taken as the research subjects; the aims were (i) to investigate the formation of iron plaque on the root surfaces of *C. cinerascens* and *P. arundinacea*, (ii) to investigate the effect of iron plaque on Pb uptake by plants, (iii) to investigate the potential role of iron plaque in distribution and translocation of Pb in the two species.

2. Materials and methods

2.1. Plant materials

C. cinerascens and *P. arundinacea* grow on islets in Poyang Lake (E115°49′–E116°46′ and N28°24′–N29°46′) (Ge et al., 2011; Zhang et al., 2013), Jiangxi Province, China. Those two species share many similar habitat and ecophysiological characteristics and show a regular distribution along a moisture gradient (Zhang et al., 2012, 2013). Plants of both study species were collected from Poyang Lake. The features of collected *C. cinerascens* plants were as follows: dry weight was 0.29 ± 0.03 g plant⁻¹, the height of the plant was 43.72 ± 2.67 cm, and number of leaves was 7-9. The features of *P. arundinacea* were as follows: dry weight was 0.32 ± 0.05 g plant⁻¹, the height of the plant was 64.81 ± 3.06 cm, and number of leaves was 8-12. The plants were stored in plastic bags in a cooler and transported to the laboratory.

2.2. Experimental design

The plants collected were pre-cultured in nutrient solution (St-Cyr and Crowder, 1989) for 3 weeks. The solution contained nutrients at the following concentrations (μ mol L⁻¹): NH₄NO₃ 500, NaH₂PO₄ · 2H₂O 60, K₂SO₄ 230, CaCl₂ 210, MgSO₄·7H₂O 160, Fe-EDTA 10, $ZnSO_4 \cdot 7H_2O$ 0.5, $MnCl_2 \cdot 4H_2O$ 0.5, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.05, H₃BO₃ 0.2 and CuSO₄ · 5H₂O 0.01. Before iron plaque induction, all plants were washed and placed in deionized water for 12 h to minimize any disturbance from other elements to iron. A solution culture experiment was carried out in a controlled greenhouse with $14/10 \,\mathrm{h}$ of day/night (light intensity, $50 \,\mathrm{\mu mol}\,\mathrm{m}^{-2}\,\mathrm{s}^{-1}$, Fluorescent lamp, Philips, type U, 18 Walt, E27). The temperature was kept at 25 °C during the day and 20 °C during the night. The relative humidity was 70–80%. Three uniform plants were transferred to a plastic pot (20 cm diameter and 25 cm height) containing 2 L nutrient solution containing 0, 30, 60, 100, 150, or $210 \,\mathrm{mg}\,\mathrm{L}^{-1}$ of ferrous iron (Fe $^{2+}$ as FeSO $_4\cdot 7H_2O$) for 7 days. The fresh nutrient solution was adjusted to pH 5.5 with 0.2 mol L⁻¹ NaOH or HCl. These treatments were called Fe0, Fe30, Fe60, Fe100, Fe150, and Fe210 respectively. After iron plaque induction, three plants were transferred to 2L nutrient solution with 10 mg L^{-1} Pb solution ((Pb²⁺ as $Pb(NO_3)_2$) for 5 days. The solution was renewed every 3 days. Each treatment had three replicates, and there were 18 pots per species. The pots were arranged randomly, and their positions were rotated regularly every day to ensure uniform conditions.

2.3. DCB extraction of iron plaque

At harvest, all roots were washed three times with deionized water (Millipore, Bedford, MA, USA). The iron plaque on the fresh root surface was extracted using dithionite-citrate-bicarbonate (DCB), as described by Taylor and Crowder (1983). Roots

were excised and incubated for 30 min at room temperature $(20-25\,^{\circ}\mathrm{C})$ in 30 mL of solution containing 0.03 mol L^{-1} sodium citrate $(Na_3C_6H_5O_7\cdot 2H_2O)$ and 0.125 mol L^{-1} sodium bicarbonate $(NaHCO_3)$, with the addition of 0.5 g sodium dithionite $(Na_2S_2O_4)$. The roots were rinsed three times with deionized water, which was added to the DCB extracts. The resulting solution was made up to 50 mL with deionized water. Concentrations of Fe and Pb in the DCB extracts were measured by ICP-AES (Optima-5300DV). The amount of iron plaque and Pb concentration in iron plaque were quantified by calculating the ratio of iron and Pb in DCB-extracts to the dry weight of the root after DCB extraction, respectively (in the unit of gram per kilogram root in dry weight, abbreviated as g kg $^{-1}$ D.W.).

2.4. Plant digestion and Pb analysis

The plant samples including roots after extraction and shoots were dried at 105 °C by desiccator (DGG-9070) for 15 min, and then dried by at 70 °C for 24 h and dry weights recorded. Samples (about 0.2-0.5 g weight) which grounded in a mill (JYL, C022E) to pass a 20 mesh sieve were digested in 5 mL concentrated HNO₃ and 2 mL 30% H₂O₂ by microwave digestion (MDS-6). A five-step digestion procedure was used: initial rise to 0.5 MPa for 3 min, 1.0 MPa for 2 min, 1.5 MPa for 5 min, and a final digestion at 2.0 MPa for 10 min (Gong et al., 2012). The digestion solutions were transferred to 50 mL volumetric flasks with deionized water (Millipore, Bedford, MA, USA) and then filtered to plastic bottles. The concentrations of Pb were determined by ICP-AES (Optima-5300DV). The concentrations of Pb in roots and shoots were quantified by calculating the ratio of Pb in roots and shoots to the dry weight of the roots after DCB extraction and the shoots, respectively (in the unit of gram per kilogram in dry weight, abbreviated as $g kg^{-1} D.W$).

2.5. Measurement of root activity

Fresh roots (about 1–2 g weight) which were cuted into 2 cm in a scissors were putted into 100 mL beaker, and 25 mL 40 $\mu g\,mL^{-1}$ α –naphthylamine and 25 mL 0.1 mol L^{-1} phosphoric acid buffer solution were added. The fresh roots were incubated for 5 min at room temperature (20–25 °C), then extracted 3 h by oscillator (ZHWY-200D). 2 mL of extracted solution, 1 mL 1% sulfanilic acid and 1 mL 100 $\mu g\,mL^{-1}$ sodium nitrite were added to 25 mL volumetric flask. The resulting solution was made up to 50 mL with deionized water and determined by UV-spectrophotometer (TU-1901).

2.6. Data analysis

The proportions of Pb in DCB-extracts, roots and shoots were calculated as follows:

 $DCB-Pb\% = (PbcontentinDCB-extracts/planttotalcontent) \times 100$

Root-Pb% = $(RootPbcontent/planttotalPbcontent) \times 100$

Shoot-Pb% = $(ShootPbcontent/planttotalPbcontent) \times 100$

The data were analyzed by Pearson correlation, two-way analysis of variance (ANOVA) procedures and compared for level of significance by Duncan's new multiple test (p < 0.05) using the statistical software SPSS19.0 (SPSS, College Station, TX, USA). Figures were generated using Sigmaplot 10.0 (Systat Software, San Jose, CA, USA). The error bars represent the standard deviation (SD) of the means.

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