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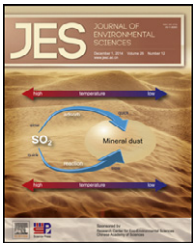

Enhanced efficiency of cadmium removal by *Boehmeria nivea* (L.) Gaud. in the presence of exogenous citric and oxalic acids

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ABSTRACT

Boehmeria nivea (L.) Gaud. is a potential candidate for the remediation of Cd contaminated sites. The present investigation aims to explore Cd tolerance threshold and to quickly identify the role of exogenous organic acids in Cd uptake and abiotic metal stress damage. Elevated Cd levels (0–10 mg/L) resulted in an obvious rise in Cd accumulation, ranging from 268.0 to 374.4 in root and 25.2 to 41.2 mg/kg dry weight in shoot, respectively. Citric acid at 1.5 mmol/L significantly facilitated Cd uptake by 26.7% in root and by 1-fold in shoot, respectively. Cd translocation efficiency from root to shoot was improved by a maximum of 66.4% under 3 mmol/L of oxalic acid. Citric acid exhibited more prominent mitigating effect than oxalic acid due to its stronger ligand affinity for chelating with metal and avoiding the toxicity injury of free Cd ions more efficiently. The present work provides a potential strategy for efficient Cd remediation with *B. nivea*.

Introduction

Industrial mining, smelting and agricultural activities such as long-term wastewater irrigation have caused prevalent soil Cd contamination and hazardous human diseases through edible plants. The persistence of Cd in soils and transfer into human body *via* food chain have aroused a major concern due to its great toxicity (Liu et al., 2008), mutagenicity, carcinogenicity and high risk to food safety. Cd contamination is extremely severe in Hunan Province (in central-south of China) because of frequent mining activities, which has led to farmland pollution, reduction of grain production and economic loss. For example, 36% of rice grown in Hunan Province was detected to have Cd levels above China's food standard regulation according to a research report (Lei et al., 2010). In order to alleviate the problem, efficient phytoremediation seems to be an appropriate route. *Boehmeria nivea* (L.) Gaud. (ramie), a perennial Urticaceae species and a primary fiber crop for textile as well as a dominant plant of mining sites in Hunan Province, has been identified as a new Cd tolerance plant species with deep root and high biomass (Wang et al., 2008). Compared to other herbaceous and woody plants, ramie has great superiority in phytoremediation of Cd contaminated sites especially the farmland for its particular capability of Cd accumulation, fast growth, ease of cultivation and propagation. All the above makes ramie not only an advantageous plant species to achieve economic and eco-environmental benefits, but also a valuable organism for studying Cd-induced physiological mechanisms to strengthen plant abiotic stress tolerance.

Due to its strong phytotoxicity, Cd can pose adverse symptoms on plants such as leaf chlorosis, browning of root tips and growth retardation, even resulting in generation of reactive oxygen species (ROS), inhibition of photosynthesis (López-Millán et al., 2009), membrane lipid peroxidation (Li et al., 2012), protein degradation, ultra-structural damage and ultimately cell death

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(Daud et al., 2009). To survive Cd-induced stress, plants have evolved protective mechanisms to mitigate and repair the oxidative impairment (Edreva, 2005), including morphological changes, physiological adaptations and antioxidative defense system. Generally, the antioxidative defense mechanisms include antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), and non-enzymatic antioxidants like glutathione (GSH), ascorbic acid (AsA) and carotenoids (Fernández et al., 2013). SOD is the key enzyme involved in catalyzing the dismutation of highly reactive superoxide radicals $O_2^{\bullet -}$ to O_2 and H_2O_2, which is further decomposed to non-toxic forms like O_2 and H_2O by APX of the ascorbate–glutathione cycle or by GPX and CAT in the cytoplasm and other cellular compartments (Jin et al., 2008).

Phytoextraction is defined as the removal of contaminants from soil by plant roots and their subsequent translocation to harvestable shoot tissues (Clabeaux et al., 2011). As an eco-friendly and cost-effective remediation technology, using plants to extract toxic metals from soil can be less destructive to soil structure and plant rhizosphere, more esthetic and more suited to impacted communities than conventional methods (Meighan et al., 2011). The extraction efficiency was closely related with contaminant availability for root uptake and translocation to the aerial parts (Bhargava et al., 2012). However, low bioavailability and limited translocation of some metals become the predominant restrictions in phytoextraction (Najeeb et al., 2011; Yang et al., 2013). Then high biomass yielding plant species with chemically assisted phytoextraction such as adding chelating agents have been used to enhance metal extraction efficiency. Chelators like low-molecular-weight organic acids (LMWOA) are capable of forming chemical complexes with metal ions and modifying the bioavailability of heavy metals in soil. LMWOA (such as citric, oxalic and malic acid), natural compounds derived from root exudates, are characterized as lower toxicity and higher biodegradability, which makes them more suitable for assisted phytoremediation than synthetic chelators.

Extensive studies have indicated that organic acids are involved in tolerance, transport and storage of heavy metal and played a key role in maintaining cellular homeostasis (Irtelli and Navari-Izzo, 2006). In particular, 25 μmol/L of citric acid could enhance Cd uptake and translocation by 61% and 2.2-fold at 4 μmol/L Cd level in *Halimione portulacoides*, respectively (Duarte et al., 2007). It could also counter Cd toxicity by improving plant growth, restoring shape and structure of root cells, and eliminating plasmolysis in *Juncus effusus* L. (Najeeb et al., 2011). Najeeb et al. (2009) found that citric acid was capable of enhancing Mn phytoextraction by 20% and alleviating metal toxicity from the reduced number of plastoglobuli in plant chloroplast. Similarly, Jean et al. (2008) reported that citric acid strengthened Cr and Ni uptake and translocation in *Datura innoxia*. Citric acid triggered more positive impact on Cu bioavailability and a visibly higher concentration of Cu uptake in tobacco shoot compared to oxalic and tartaric acid (Evangelou et al., 2006). Comparatively, citric acid (10 mmol/L) demonstrated a higher Cu mobilization (from 1 in the control to 42 mg/kg) than tartaric acid (Pérez-Esteban et al., 2013). These studies put forward an insight into the positive effect of exogenous organic acids on plant biomass, metal uptake and translocation, and metal tolerance for improving phytoremediation efficiency. In this work, a new Cd tolerant plant was studied to extend the range of plant species for better understanding of its potential application in phytoremediation.

Previous studies mainly focused on the accumulation, transport and tolerance mechanisms of ramie and subcellular distribution of heavy metals in plant tissues (Liu et al., 2007; Xia et al., 2009; Wang et al., 2008, 2011). However, to our best knowledge, little information is available on the effect of LMWOA on Cd phytoextraction and tolerance. Therefore it seems significant to demonstrate the natural chelator–Cd–plant interaction in this species. The objectives of this study were to: (1) explore the potential of ramie in phytoextraction of Cd; (2) compare the effect of different concentrations of exogenous citric and oxalic acids on plant growth, Cd uptake and translocation; and (3) determine the role of citric and oxalic acids in Cd tolerance of ramie by regulating the antioxidant defense system involved in stress endurance.

1. Materials and methods

1.1. Plant collection and hydroponic culture

A batch of ramie seedlings was collected from Ramie Research Institute (Changsha, Hunan Province, China). Plants were acclimatized to hydroponic condition in 1/8 Hoagland nutrient solution (pH 6.0 ± 0.5) for 2 weeks. The solution was aerated continuously and renewed every 2 days. The experiments were conducted in a growth chamber with 25/20°C, day/night temperature, 60%–70% relative humidity and 14 hr photoperiod at light intensity of 300 μmol/(m^2·sec).

1.2. Cadmium treatment and the addition of organic acids

After preculturing for 2 weeks, the plants were treated in triplicates in a completely randomized design as follows: 0 (Cd_0), 2 (Cd_2), 5 (Cd_5), 10 (Cd_{10}) mg/L Cd concentrations as $Cd(NO_3)_2$ and Cd_{10} + CA_1, Cd_{10} + CA_2, Cd_{10} + OA_1, Cd_{10} + OA_2 (CA_1 and CA_2 refer to 1.5, 4 mmol/L citric acid; OA_1 and OA_2 refer to 3, 9 mmol/L oxalic acid, respectively). Organic acids were applied at highest Cd level (10 mg/L) after Cd exposure for 1 week. Plant tissues were harvested after one month. Fresh samples were frozen immediately in liquid nitrogen and stored at −80°C for subsequent analysis.

1.3. Analysis of cadmium uptake

Plant roots and shoots were dried at 80°C till constant weight. About 0.5 g dry samples were digested with 10 mL HNO_3 and 3 mL $HClO_4$ at 160°C by heating to be transparent. Digested liquid was washed several times into a 100 mL volumetric flask and diluted with de-ionized water to the calibration line for measurement. The Cd content was determined by atomic absorption spectrometer (Analyst 700, PerkinElmer, Massachusetts, USA) and calculated as mg/kg of dry weight (dw). Translocation factor (TF) is determined as the ratio of heavy metal concentration in plant shoot to that in root.

1.4. Plant growth and root activity analysis

Fresh weight (fw) and dry weight of plant were measured by electronic balance as an indicator of plant growth. The water content (C_w, %) was determined on the basis of Eq. (1):

$$C_w = \frac{W_f - W_d}{W_f} * 100\% \quad (1)$$

where, W_f (g) and W_d (g) refer to fresh weight and dry weight of plant, respectively.

Root activity (RA) was determined by the triphenyl tetrazolium chloride (TTC) method (Clemensson-Lindell, 1994). Fresh roots (about 0.5 g) were immersed in beakers with the addition of 10 mL of 0.4% TTC and phosphate buffer (1/15 mol/L, pH 7.0). After incubation at 37°C for 2 hr in the dark, 2 mL of 1 mol/L H_2SO_4 was added to terminate the reaction. Then the samples