Ecotoxicology and Environmental Safety 145 (2017) 200–206

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Differential effects of citric acid on cadmium uptake and accumulation between tall fescue and Kentucky bluegrass

ShuTing Wang, Qin Dong, ZhaoLong Wang*

School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200240, PR China

ARTICLE INFO

Keywords:
Citric acid
Cadmium
Uptake
Accumulation
Tall fescue
Kentucky bluegrass

ABSTRACT

Organic acids play an important role in cadmium availability, uptake, translocation, and detoxification. A sand culture experiment was designed to investigate the effects of citric acid on Cd uptake, translocation, and accumulation in tall fescue and Kentucky bluegrass. The results showed that two grass species presented different Cd chemical forms, organic acid components and amount in roots. The dormant Cd accumulated in roots of tall fescue was the pectate- and protein- integrated form, which contributed by 84.85%. However, in Kentucky bluegrass, the pectate- and protein- integrated Cd was only contributed by 35.78%, and the higher proportion of Cd form was the water soluble Cd-organic acid complexes. In tall fescue, citric acid dramatically enhanced 2.8 fold of Cd uptake, 3 fold of root Cd accumulation, and 2.3 fold of shoot Cd accumulation. In Kentucky bluegrass, citric acid promoted Cd accumulation in roots, but significantly decreased Cd accumulation in shoots. These results suggested that the enhancements of citric acid on Cd uptake, translocation, and accumulation in tall fescue was associated with its promotion of organic acids and the water soluble Cd-organic acid complexes in roots.

1. Introduction

Cadmium (Cd) is a highly toxic and non-essential element to all living organisms. Soil Cd contents have dramatically increased in recent decades through mining, smelting, electroplating, wastewater irrigation, and abuse of chemical fertilizers and pesticides (Ghosh and Singh, 2005; Ingwersen and Streck, 2006; Chen et al., 2013). Soil Cd contamination become a serious worldwide problem to crop growth, food production, and human health (Cai and Braids, 2002; Zhou and Song, 2004; Franz et al., 2008).

Phytoremediation is an environmental friendly technique to cleanup contaminants in the soil and groundwater (Salt et al., 1998; Wan et al., 2016) and can potentially be used to reduce contamination of Cd and other heavy metals (Ghosh and Singh, 2005). The first and fundamental step for phytoremediation is the availability and uptake of soil contaminants by plant roots. Many studies demonstrated that plant roots are able to excrete organic acids into rhizosphere to enhance metal solubility and availability and facilitate their uptake by roots (Cieslinski et al., 1997; Vamerali et al., 2010). Citric acid is one of the common organic acids excreted from plant roots (Cieslinski et al., 1997; Montiel-Rozas et al., 2016). Krishnamurti et al. (1995) found that Cd-citric acid complex-bound contributed significantly to the bioavailable Cd in the soil. Some researchers found that citric acid significantly alleviated Cd uptake and accumulation in roots and shoots of plants (Ehsan et al., 2014). Arsenov et al. (2017) found that exogenous citric acid enhanced antioxidant defense and phytoextraction of Cd in willows (*Salix* spp.). Citric acid excreted from the roots significantly enhanced bioaccumulation of heavy metals, suggesting that citric acids can be considered natural chelating agents to enhance phytoextraction (Naidu and Harter, 1998).

Organic acid is also reported to be present as complexes to heavy metals in plant cells. Metal organic acids interrelationships in plants may also play an important role in the metal transportation, cell compartments, and plant tolerance. Organic acids were combined with heavy metal ions in the xylem sap for their translocation from roots to shoots (Tatar et al., 1999; Mnasri et al., 2015).

Beside their contribution to heavy metal translocation, organic acids may also be involved in metal detoxification through cheating processes leading to reduction of the free ionic forms of metals which are by far the most toxic forms. It is suggested that the build-up in shoot citrate concentrations could be positively correlated with plant capability to detoxify and accumulate Cd in several plants species (Krämer et al., 2000; Sun et al., 2006; Ghnaya et al., 2013). Pence et al. (2000) found that *Thlaspi caerulescens* plant synthesized more organic acids when subjected to Cd^{2+} in order to reduce the reactivity of free Cd^{2+} ions with proteins thus allowing a high accumulation of Cd in the shoots

* Corresponding author.
E-mail address: turf@sjtu.edu.cn (Z. Wang).

http://dx.doi.org/10.1016/j.ecoenv.2017.07.034
Received 7 March 2017; Received in revised form 3 July 2017; Accepted 15 July 2017
Available online 19 July 2017

without injury symptoms. Mnasri et al. (2015) showed that high citric acid accumulated in xylem sap and shoot could be involved in Cd chelation and contributed the Cd tolerance in *Sesuvium portulacastrum*.

However, hyperaccumulators with hyper-capacity of tolerance and accumulation of heavy metals could be related to their different strategies in root uptake and root to shoot translocation. Zhan et al. (2016) found that the soil available Cd content was significant negatively correlated to the contents of citric acid and malic acid in roots of *Vicia faba*. Meier et al. (2012) found that the roots of Cu-adapted/tolerant metallophyte species excreted higher amounts of organic acids and restricted metal acquisition by plants.

Our previous studies showed that tall fescue and Kentucky bluegrass could uptake and accumulate 44.5 and 40.8 fold more Cd than Cd hyperaccumulator, *Solanum nigrum*, without any toxic symptoms (Xu and Wang, 2014). These two grasses showed differential capacity of Cd uptake by roots and translocation from roots to shoots (Xu and Wang, 2013; Dong et al., 2017). However, it is still unclear whether the differential Cd accumulation was regulated by the root organic acids in these two turfgrass species. Therefore, the objectives of this study were to investigate: 1) the relationship of root organic acids and Cd uptake and accumulations, and 2) the effects of citric acid on Cd rhizosphere availability, root uptake, and accumulations between tall fescue and Kentucky bluegrass.

2. Materials and methods

2.1. Plant materials and growth conditions

A sand culture experiment was designed to investigate the differential effects of citric acid on Cd uptake and accumulation between tall fescue (*Festuca arundinacea*) and Kentucky bluegrass (*Poa pratensis*). Seeds of tall fescue (cv 'Barlexas') and Kentucky bluegrass (cv. 'Midnight') were sown in plastic pots (165 mm length, 165 mm width, 160 mm height) with five small holes at the bottom to ensure good drainage. Plastic pots were filled with washed quartz sand (5.0 kg each, sand was sieved in particle size between 0.25 and 0.50 mm) as seeding matrix with gauze in the bottom to prevent sand from escaping through drainage holes. Water appropriately to maintain a moist soil and a good seedling emergence. After 3 months' growth, the mature plants were moved into a phytotron with canopy photosynthetically active radiation (PAR) of 600 μmol•m^{-2}•s^{-1} and an average day/night temperature of 28/16 °C. Hoagland solution (250 ml each) was applied for nutrient supply every week. All plants were watered with tap water every 2 days in order to maintain 75–85% of sand moisture. After 2 weeks' adaptation period, plants were subjected to experimental treatments.

2.2. Treatment and experiment design

The experiment was arranged in a randomized complete block design with four replicates. Three treatments were: i) the control (no Cd); ii) Cd treatment (100 mg Cd kg^{-1}); iii) Cd + CA treatment (100 mg Cd kg^{-1} + 1 mmol kg^{-1} citric acid) for tall fescue and Kentucky bluegrass. $CdCl_2$ was applied by uniformly injecting 250 ml of $CdCl_2$ solutions into the sand of each pot. Citric acid was added by injection into the sand at 5 days' interval after 3 days of Cd application. During the experiment, plants were fertilized once a week with 200 ml Hoagland's nutrient solution per pot, and watered by adding water in the bottom trays under plastic pots every 2 days to maintain the sand moisture at 75–85% and to avoid Cd^{2+} leaching. After 30 days of treatments, plants were harvested for the following measurements.

2.3. Measurement

2.3.1. Plant height and biomass

After 30 days of treatment, the vertical height of tall fescue and Kentucky bluegrass was measured by a ruler. Ten plants in each pot were measured for plant height and the average was represented as the pot.

After the measurement of other parts, all the plants were dug out and washed clean by de-ionized water, then dried with absorbent paper. Shoots and roots were separated and dried at 100 °C for 10 min, then oven-dried at 80 °C to a constant weight and the dry weight was recorded as biomass.

2.3.2. Extraction Cd in different chemical forms in roots and shoots

Cd associated with different chemical forms in plant tissues was extracted by designated solutions in the following order (Wu et al., 2005; Xu and Wang, 2014; Dong et al., 2017): (i) 80% ethanol, extracting the water soluble inorganic Cd, including nitrate/nitrite, chloride, and aminophenol Cd; (ii) deionized water (d-H_2O), extracting the water-soluble Cd-organic acid complexes and $Cd(H_2PO_4)_2$; (iii) 1 M sodium chloride (NaCl), extracting the pectate and protein integrated Cd; (iv) 2% acetic acid (HAc), extracting the undissolved Cd phosphates including $CdHPO_4$ and $Cd_3(PO_4)_2$; (v) 0.6 M hydrochloric acid (HCl), extracting the Cd oxalate; (vi) the rest was in forms of resides.

Plants were separated into shoots and roots. The roots were immersed in 20 mmol EDTA-Na_2 solution for 15 min to remove Cd adhering to the root surface (Xu and Wang, 2014), washed with deionized water, and then dried with absorbent paper. Fresh leaves and roots (2.5 g each) were cut into 1 cm long clippings, and ground in liquid nitrogen. Then plant tissues were transferred into a 50 ml centrifuge tube, added into 37.5 ml extraction solution. The mixture was incubated at 30 °C for 18 h. Then, the extract was centrifuged at 5000 g for 10 min. The first supernatant was transferred into a 50 ml flask bottle. The second 37.5 ml extraction solution was added to the centrifuge tube for another 2 h, and the supernatant was also reclaimed in the 50 ml flask bottle. The procedure was repeated twice in the next 4 h. The final 150 ml extraction solution was collected and evaporated to constant weight, and digested in supra-pure concentrated HNO_3 and $HClO_4$ (4:1, v/v) at 130–150 °C, diluted to 25 ml. The plant tissues retained in the centrifuge tube were subjected to the next extraction solution with the same extraction procedures. At the end of the sequential extraction, the residues were evaporated to constant weight and digested in supra-pure concentrated HNO_3 and $HClO_4$ (4:1, v/v) at 130–150 °C, diluted to 25 ml. The concentrations of Cd were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 8000, PerkinElmer, America).

2.3.3. Cd in different chemical forms in rhizosphere soil

Rhizosphere soil samples were carefully collected by removing the sand adhering to the plant roots. Cd species in rhizosphere soil were divided into 4 fractions (i.e., acid-extractable, reducible, oxidizable and residual fractions) using a four-stage BCR approach of sequential extraction procedures according to Huang et al. (2014). The extraction reagents and conditions of the BCR approach were briefly described as the following steps. The concentrations of Cd in each of fractions were measured with AAS as described above.

Step 1: Acid soluble. 1.5 g sand soil was mixed with 20 ml 0.11 mol L^{-1} CH_3COOH, then shaken 16 h at 23 °C, centrifuged at 4000 g for 20 min. The supernatant was transferred into a 50 ml flask bottle for acid-extractable Cd. The residue was used for extraction in next step.

Setp 2: Reducible. 20 ml 0.5 mol L^{-1} hydroxylamine hydrochloride (pH1.5) was added to the previous residue. The mixture was shaken 16 h at 23 °C, then centrifuged. The supernatant was transferred into a 50 ml flask bottle for reducible Cd and the residue was used for the next extraction.

Step 3: Oxidizable. The residue was incubated with 5 ml 8.8 mol L^{-1} H_2O_2 1 h at 23 °C, then 1 h at 85 °C. Another 5 ml 8.8 mol L^{-1} H_2O_2 was added and the mixture was shaken 1 h at 85 °C. After adding 25 ml 1 mol L^{-1} NH_4COOCH_3, the mixture was shaken 16 h at 23 °C, then centrifuged. The supernatant was transferred into a