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Distribution and speciation of chromium accumulated in *Gynura pseudochina* (L.) DC.

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ABSTRACT

Soil and water contamination with chromium is an issue of recent concern in Thailand due to increases in industrial activity. *Gynura pseudochina* (L.) DC., a chromium tolerance plant, could be employed to address this problem via phytoremediation. To understand the tolerance mechanism, this study investigated the speciation and distribution of chromium accumulated in *G. pseudochina* (L.) DC. using AAS, XAFS, μ-XANES, μ-XRF imaging and EPR. The plants were separately treated with $K_2Cr_2O_7$ and $Cr_2(SO_4)_3$ in a hydroponic system. μ-XRF imaging clarified the distributions of Cr, Fe, Zn, Ca, Cl, K and S within the samples. In *G. pseudochina* (L.) DC. treated with Cr(VI) solution, the Cr was mainly distributed in the vascular bundle and periderm of the tuber, the stem xylem, the vein and the epidermis, including the trichome of the leaf tissues. This Cr distribution corresponded to those of Cu, Fe and Zn. In *G. pseudochina* (L.) DC. treated with Cr(III) solution, the Cr was distributed in the periderm of the tuber, the stem cortex, and the epidermis and parenchyma of the leaf tissues. μ-XANES and XAFS indicated that highly toxic Cr(VI) was reduced to the intermediate Cr(V) and accumulated as less toxic Cr(III), and EXAFS spectra showed that the reduced Cr(III) was bound to oxygen ligands. The coordination number (*N*) and the interatomic distance (*R*) to the first shell were approximately 3–4 (*N*) and 2 Å (*R*), respectively. EPR spectra of the plant samples treated with Cr(VI) revealed the presence of Cr(V) and Cr(III). Thus, Cr(III) and Cr(VI) were taken up into the vascular system and transported from the roots to the leaves. Cr(III) was distributed via the symplast system to the ground tissue and accumulated mainly in the stem cortex. Cr(VI) was transported to the xylem via the apoplast system, and the adsorption of Cr(VI) and its reduction to Cr(V) and Cr(III) occurred on oxygen ligands in the lignocellulosic structure of the xylem and vein.

1. Introduction

Chromium (Cr) is used in a variety of industrial applications, such as leather processing and finishing, the production of refractory steel, drilling muds, electroplating cleaning agents, catalytic manufacturing and the production of chromic acid and specialty chemicals. Hexavalent chromium (Cr(VI)) compounds are used in industry for metal plating, cooling tower water treatment, hide tanning and wood preservation. These activities have caused widespread Cr contamination in the environment and have increased its bioavailability and biomobility (Kotas and Stasicka, 2000; Shanker et al., 2005). Cr toxicity depends on the valence state. Hexavalent chromium (Cr(VI)) is more highly toxic and mobile than trivalent chromium (Cr(III)) (Nieboer and Jusys, 1988; Shanker et al., 2005). Soil and water contamination with chromium is an issue of recent concern in Thailand, a developing country, due to increases in industrial activity. A notification from the National Environmental Board No. 25, B.E. 2547 (2004), setting the soil quality standards for habitat and agriculture, states that Cr(VI) in soil must not exceed 300 mg kg^{-1}. A notification from the Ministry of Science, Technology, and the Environment No. 3, B.E. 2539 (1996), setting the industrial effluent standards, states that Cr(III) and Cr(VI) in water discharge must not exceed 0.75 and 0.25 mg L^{-1}, respectively.

Phytoremediation is the process through which land contamination is ameliorated by growing plants that are able to remove contaminating chemicals from the environment and accumulate them in a non-phytotoxic form (Suresh and Ravishankar, 2004). In the case of heavy metal contamination, phytoremediation involves the growth of plants that possess metal tolerances and can accumulate high concentrations of metal from the contaminated area. Phytoremediation is an environmentally friendly and cheap process that does not requires a large amount of equipment or labour. Although it is relatively slow, this process is easy to carry

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out, and sites can be cleaned without removing the polluted soil (Pilon-Smits, 2005). Moreover, the plant hyperaccumulator can be harvested, and the collected metal can be extracted (Garbisu and Alkorta, 2001).

Gynura pseudochina (L.) DC., a tuber plant belonging to the Asteraceae Family, was found growing in a zinc mine and has a potential use in phytoremediation of zinc and cadmium (Phaenark et al., 2009). Our preliminary study found that *G. pseudochina* (L.) DC. was also able to accumulate chromium and reduce toxic Cr(VI) to less toxic Cr(III). However, the plant's mechanisms for accumulation and detoxification of the chromium should be clearly understood before application. Therefore, this paper aims to study the accumulation, distribution and speciation of chromium in *G. pseudochina* (L.) DC. treated with Cr(VI) and Cr(III) using atomic absorption spectroscopy (AAS), micro X-ray fluorescence (μ-XRF) imaging, micro X-ray absorption near-edge structure (μ-XANES), X-ray absorption fine structure (XAFS), and electron paramagnetic resonance (EPR) spectroscopy.

2. Methods

2.1. Plant materials

Tubers of *G. pseudochina* (L.) DC. were derived from an area without chromium contamination in Tak Province, Thailand. The tubers were propagated in a commercial, fertile soil obtained from Nongporn Co. Ltd., Thailand; 20% C, 2.3% H, 0.44% N, 0.14% S and no Cr contamination, for six months. The new propagated tubers were cleaned and cut into pieces weighing 5–6 g. Each tuber was grown in a pot containing 300 g of sterilised sand (soil-less cultivation). Plants were watered with a modified Hoagland's nutrient solution in deionised water. The composition of 1 L of the nutrient solution consisted of 5 ml of 1 M KNO_3, 1.0 ml of 1 M $NH_4H_2PO_4$, 5 ml of 1 M $Ca(NO_3)_2{\cdot}4H_2O$, 2.0 ml of 1 M $MgSO_4{\cdot}7H_2O$, 2.9 ml of 1 g L^{-1} H_3BO_3, 1.8 ml of 1 g L^{-1} $MnCl_2{\cdot}4H_2O$ and 1.0 ml of 0.5% (w/v) Fe-EDTA. Plants were grown for one month in a glass house at 20–40 °C, 70–75% humidity and >10,000 lux light intensity at noon. The one-month-old plants were separately treated with Cr(VI) and Cr(III) solutions (100 mg L^{-1}, pH 5.5 ± 0.5), which were prepared from potassium dichromate ($K_2Cr_2O_7$) and chromium sulphate ($Cr_2(SO_4)_3$), respectively, in a hydroponic system. Only the root was placed in the solution for a period of two weeks. Control plants were treated with deionised water at pH 5.5 ± 0.5. The plant samples were harvested before being separated into tuber (medullar and periderm), stem and leaves for analysis with AAS, μ-XRF imaging, XAFS and EPR.

2.2. Chromium accumulation in plants

The plants treated with 100 mg L^{-1} of Cr(VI) solution for two weeks were harvested and washed with an excess of running deionised water, and then the plants were separated into the tuber and shoots (leaves and stem). The wet weight of each part of the plant was measured before the parts were dried at 80 °C for 24 h. Each sample was then ground into a homogenous powder. Each sample (0.250–0.500 g) was digested using the modified method of Miller (1998). The steps consisted of digesting with 3 ml of conc. HNO_3 (65% v/v), heating at 150 °C for 1 h, then adding 1 ml of $HClO_4$ (70% v/v), heating again at 215 °C for 2 h, followed by the addition of 3 ml of deionised water and finally heating at 90 °C for 1 h. The digestion solution was filtered through a Whatman No. 542 filter paper. The volumes of the filtrates were adjusted to 10 ml with deionised water before analyses were conducted to determine the chromium concentration via atomic absorption spectroscopy (AAS) (Shimadzu AA-680, Japan).

2.3. μ-XRF imaging and μ-XANES and XAFS analyses

Chromium-treated plants were subjected to two-dimensional μ-XRF imaging and XAFS analysis. After two weeks of Cr(VI) and Cr(III) treatment, the plant samples were harvested and washed with an excess of running deionised water, and then the plants were separated into the tuber, leaves and stem. A thin section of the freeze-dried sample was prepared to maintain its tissue structure and chemical state. The cross sections of the tuber, stem and leaves were cut to a thickness of 200–300 μm using a vertical slicer (HS-1 JASCO, Japan) and immediately placed on dry ice. The sections were freeze-dried overnight using a lyophiliser (Labconco Lyph Lock 6 Freeze Dryer, USA). For bulk XAFS analysis, an amount of each freeze-dried plant part (tuber (medulla and periderm), stem and leaves) was ground, pressed into a pellet and then sealed in a Mylar plastic bag (Mylar polyester film, No.100, Chemplex, USA). Reference materials utilised for Cr(III) included $Cr(NO_3)_3$, $CrCl_3$, Cr_2O_3, $Cr_2(SO_4)_3$ and CrS; Cr(V) reference materials included $Na[Cr(O)(ehba)_2]{\cdot}H_2O$; and Cr(VI) reference materials included CrO_3 and $K_2Cr_2O_7$. The CrS and $Na[Cr(O)(ehba)_2]{\cdot}H_2O$ were synthesised following the methods of Jellinek (1957) and Krumpolc and Rocek (1980), respectively; other analytical grade reference materials were purchased from Sigma and Fluka. μ-XRF imaging and XAFS analysis were performed at beamline 4A and beamline 12C, respectively, at the Photon Factory (PF), High Energy Accelerator Research Organisation (KEK) (Tsukuba, Japan). The experimental conditions for μ-XAF imaging and XAFS analysis are summarised in Table 1. The XAFS analysis data were analysed using Rigaku Rex2000 Version 2.3.2.

2.4. EPR measurements

The plant samples treated with Cr(VI) for two weeks were harvested and washed with an excess of running deionised water, and then the plants were separated into the tuber (medulla and periderm), leaves and stem. The separated samples were freeze dried and then ground into a powder consisting of 75–100 μm particles. The powdered samples were weighed and transferred into 3 mm i.d. quartz tubes for EPR spectral measurements. All of the EPR spectra were acquired on a JEOL EPR spectrometer (JES-RE2X, Japan) at X-band frequencies using Gunn diodes as the microwave source. Full spectra were obtained at ambient temperature (*ca.* 25 °C) as first derivatives of the microwave absorption; the operating conditions were as follows: microwave power, 1 mW; microwave frequency, 9.43 GHz; centre field, 260 mT; modulation amplitude, 100 kHz; sweep time, 2 min; sweep width, 2.5 × 100 mT; amplitude, 2.5 × 10; and time constant, 0.03 s. Spectra of a specific area were performed at a centre field of 340 mT, a sweep time of 0.5 min, and a sweep width of 1.5 × 10 mT. *g*-values were obtained using diphenylpicrylhydrazyl (DPPH) as an external reference standard ($g = 2.0036$).

3. Results and discussion

3.1. Chromium accumulation

G. pseudochina (L.) DC. was treated with 100 mg L^{-1} of Cr(VI) solution for two weeks in the hydroponic system. The Cr accumulated in the tuber and shoots (leaves and stem) weighed 823.1 ± 5 mg kg^{-1} and 787.9 ± 8 mg kg^{-1}, respectively. The Cr concentration in the tuber was higher than that in the shoots (leaves and stem). A translocation factor (TF), i.e., the ratio of the element's concentration in shoot tissue to the element's concentration in root tissue, estimates the translocation efficiency of a plant. The TF was determined according to Eq. (1) (Gheju et al., 2009). The