Phytoremediation of chromium: distribution and speciation of chromium in *Typha angustifolia*

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Abstract

Chromium (Cr), especially in hexavalent chromium [Cr(VI)] may contaminate water or soil and cause detrimental effects, as it is potentially carcinogenic and teratogenic. Phytoremediation using plants such as *Typha angustifolia* provides an alternative approach for handling Cr waste. The objective of this study was to determine the mechanism of Cr accumulation in *T. angustifolia*. Hydroponic media containing *T. angustifolia* was added with 0, 1, 5, 10 and 20 ppm of Cr (VI) ($K_2Cr_2O_7$). After 15 days of treatment, distribution and speciation of Cr in roots and shoots of *T. angustifolia* were analyzed using XAS and µ-XRF. Results showed that Cr was detected in almost all parts of root and shoot at different intensities. Intensities of Cr was higher in roots (especially in the vascular bundle) than in shoot. Cr speciation in the root and shoot was found as trivalent chromium [Cr(III)] which formed as a result of Cr(VI) reduction. Based on the patterns of Cr distribution and speciation, results of this study suggest that *T. angustifolia* in this study does not reduce Cr(VI) to become Cr(III) inside the plants.

Introduction

EtOAcChromium (Cr), especially in the hexavalent form [Cr(VI)], is the second most commonly found waste element in the world, after arsenic (Ar).[1] Approximately 90% of this waste originates from metallurgical industries, 5% from refractories and foundries, and 5% from other chemical industries.[2] The level of Cr toxicity depends on its valence, *i.e.,* Cr with a valence of six [Cr(VI)] is more toxic than Cr with a valence of three [Cr(III)].

EtOAcIndonesia as a developing country has 78,221 sites with possible exposure to Cr(VI).[3] This statement is in line with data from the Ministry of Industry-Indonesia which describes a 7% increase in industrial development each year.[4] Therefore, it is expected that the production of waste containing Cr will continue to increase and contaminate the environment, especially soil and water. Consequently, people living in certain areas of Indonesia face high possibility of health risks such as infertility, respiratory problems and birth defects which are caused by Cr(VI).[3]

Efforts to reduce and remove environmental contaminants have so far included the processes of precipitation, electrolysis, filtration, ion exchange and phytoremediation. However, phytoremediation is perhaps the least expensive, easiest, and most environmentally friendly approach. Phytoremediation is the technology of using plants to reduce or remove contaminants from the environment and accumulating them within the plant.[5,6] Not all plants can be used as phytoremediation agents because not all of them have the required defense mechanisms against contaminants, or the ability to accumulate contaminants in less toxic forms. At present, only 450 Angiosperms are known to be heavy metal hyperaccumulators.[7]

EtOAc*Typha* is a plant genus from the Typhaceae family which is commonly used in constructed wetland systems designed to reduce and remove contaminants. Certain *Typha* species, such as *T. angustata* and *T. latifolia* are found to accumulate Cr in their roots and shoots.[8,9] It is suggested that another species, *T. angustifolia* which is easily found in Java, Indonesia has also potential to accumulate Cr. However, more research is needed to understand the mechanism involved in Cr accumulation, specifically in *T. angustifolia*. Therefore, the objective of this research was to study the mechanism of Cr accumulation based on its distribution and speciation in *T. angustifolia* treated with Cr(VI) using micro X-ray fluorescence (µ-XRF) imaging and X-ray absorption near-edge structure (XANES).

Materials and Methods

Plant material

EtOAcAs many as 60 *T. angustifolia* plants were collected from areas free from Cr contamination at Eretan Kulon - Indramayu, West Java, Indonesia (6°18'44.19"S and 108°02'39.93"T; Figure 1). This research only used plants aged 2.5 – 3 months old. The plants were first cleaned using tap water to remove soil and clay, then acclimated in water containing commercial hydroponic nutrients (AB mix). The composition of 1.6 ppm nutrient solution consisted of $5Ca(NO_3)_2.NH_4NO_3.10H_2O$, KNO_3, Fe-EDTA, KH_2PO_4, $(NH_4)_2SO_4$, K_2SO_4, $MgSO_4.7H_2O$, $MnSO_4.4H_2O$, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, H_3BO_3, and $(NH_4)6Mo7O_{24}.4H_2O$. Plants were acclimated for two weeks in a greenhouse at 28-35°C, 51-70% humidity and 55.7-107.8 KLux light intensity at noon.

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Key words: Chromium; phytoremediation; distribution; speciation; *Typha angustifolia*.

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Chromium phytoremediation treatments

EtOAcThe plants were treated in 1, 5, 10, and 20 ppm of Cr(VI) from $K_2Cr_2O_7$ (Merck) for 15 days with four replications. Every treatment contained three plants with a total wet weigh of 380-400 g. The plants were then harvested after 15 days treatment and washed using tap water and deionized water. Harvested plants were separated into root and shoot, weighed for wet biomass, and then dried at 80°C for 48 hours. Afterwards, samples were digested by wet ashing method and measured for Cr accumulation using atomic absorption spectroscopy (AAS) with 357.9 nm to decide the preliminary and early (before the treatment) Cr content in the plant.[10]

μ-XRF imaging and XANES analysis

EtOAcFor μ-XRF imaging, the root and shoot of plants were cut into small parts (measuring about 1 cm), placed in an acrylic sample holder, and then placed into μ-XRF detection. Dwell time per point was five seconds and step size between points was 0.05 mm. For XANES, roots and shoots were cut into 2 cm pieces, put on kapton tape then placed in a sample holder for XAS detection. This study used the upper shoot part was examined. Reference materials utilized were $K_2Cr_2O_7$ and CrO_3 for Cr(VI); and Cr_2O_3 for Cr(III). The μ-XRF imaging and XANES analysis were performed at beamline 6b and 8 in Synchrotron Light Research Institute at Nakhon Ratchasima, Thailand. The μ-XRF imaging and XANES data were analyzed using ROI imaging tools and ATHENA.[11]

Results

EtOAcThe Cr content in plants was also measured before treatments were started. Early measurements showed that the plants had 0 mg/kg total Cr, indicating that the plants were free from Cr contaminant. In a preliminary experiment, total Cr in root and shoot was analyzed using AAS. Results showed that Cr concentrations were detected in both root and shoot of *T. angustifolia* treated with 1, 5, 10 and 20 ppm of Cr(VI). However, preliminary AAS measurements indicated that the accumulation of Cr in roots (287.16 to 4399.79 mg/kg) was significantly higher than in the shoots (234.02 to 1157.28 mg/kg) (P>0.05) (Vidayanti and Choesin 2015, unpublished data).

Distribution of Cr

EtOAcThe XRF intensity was normalized by incident X-rays and expressed with a color scale from blue to red that corresponded from lowest to highest, respectively (Figures 2 and 3). μ-XRF imaging from roots treated with Cr(VI) is shown in Figure 2. Cr was detected throughout all parts of the primary root, but was primarily distributed in the center part which consists of the vascular bundle, and decreased towards the outer part. It was clearly indicated that concentration of Cr in the vascular bundle was higher than in other parts. It could be assumed that Cr was transported or translocated into the shoot through the vascular bundle, *i.e.,* xylem. Cr uptake by roots could also be translocated into other tissues and organelles. During upward translocation process, Cr may be transferred to the xylem through the apoplastic route. We assumed that the symplastic route to the cortex and pith may have occured. This process must be confirmed by further root cross-section analysis.

EtOAcT. angustifolia is a wetland plant which has certain adaptive structures not found in most terrestrial plants, including aerenchyma in its shoot. Similar to observation in roots, Cr was also detected and distributed in almost all parts of the shoot. Upwards translocation process of Cr from

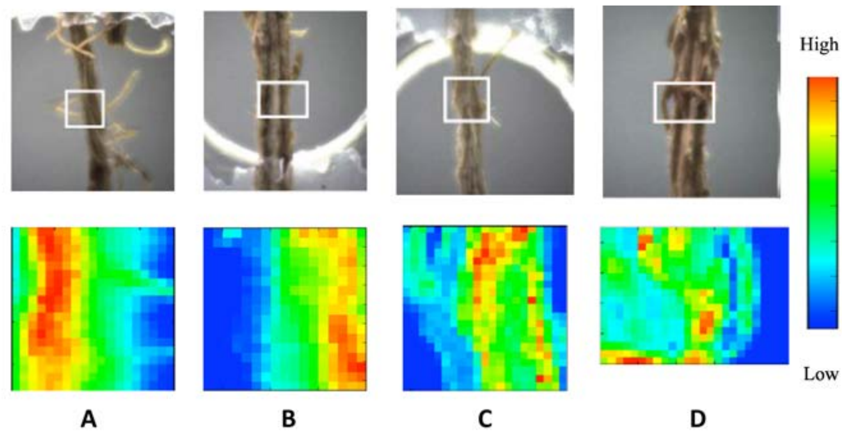

Figure 1. μ-XRF imaging of *T. angustifolia* roots treated with Cr(VI) (A) 1 ppm (B) 5 ppm (C) 10 ppm and (D) 20 ppm. Photographs of the root treated with Cr(VI) (A) 1 ppm (B) 5 ppm (C) 10 ppm and (D) 20 ppm.

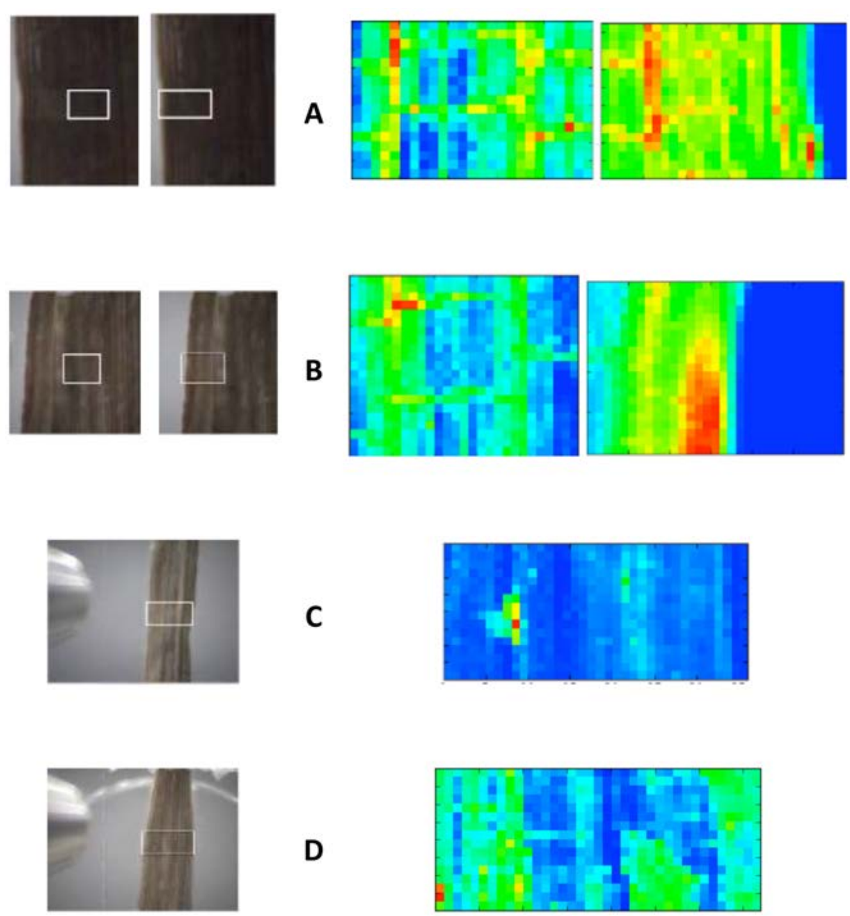

Figure 2. μ-XRF imaging of *T. angustifolia* shoots treated with Cr(VI) (A) 1 ppm (B) 5 ppm (C) 10 ppm Ad (D) 20 ppm. Photographs of the shoot treated with Cr(VI) (A) 1 ppm (B) 5 ppm (C) 10 ppm and (D) 20 ppm.

root to shoot may occur through the xylem and veins but cross section observations of the root are needed to confirm this. The distribution pattern of Cr in shoots was not as clear as that observed in roots. Cr was initially expected to accumulate in specific parts of the shoot to avoid toxic elements in photosynthetic active tissue.

Speciation of Cr

EtOAcXANES spectra of samples were compared and fitted using spectra of Cr(VI) and Cr(III) reference materials which has energy peaks in 6001-6020 eV and 6021-6040 eV. Figure 3 was obtained from Cr K-edge XANES spectra of root and shoot. At first, Cr species between root and shoot was expected to be different, and a difference was also expected among Cr concentration treatment. However, it was clearly illustrated that the oxidation state of accumulated Cr in plants treated with Cr(VI) was Cr(III).

EtOAcThe curve pattern and peak of samples was similar with the peak of Cr(III) reference (Figure 3). XANES fitting of the spectra for the root of plants treated with Cr(VI) exhibited features mainly consisting of Cr(III). This Cr species was also found in shoots. These results suggest that *T. angustifolia* did not reduce Cr(VI) in its roots. This plant uptook Cr(III) directly from the medium, so we suggested that Cr(VI) was reduced to Cr(III) in the media before being uptake by roots. Afterward, Cr would be transported to shoot, where it is accumulated as Cr(III). That is why XANES only detected Cr(III) in the shoot.

Discussion

EtOAcCr is not known as an essential or important element for plant growth. High concentrations of Cr may cause detrimental effects on plants, ranging from chlorosis to death. However, small amounts of Cr (5 mg/kg) can increase nitrification rates in soil and increase the amount of nitrogen in plants.[12] For plants that are able to accumulate and tolerate heavy metals, high concentrations of heavy metal in their environment will not detrimentally affect their survival. These plants are able to absorb heavy metals from the environment and accumulate them by distributing to other plant parts.

EtOAcThe concentration of Cr in roots observed in this study was distributed primarily in the center part, which corresponds to vascular bundles containing xylem and phloem and have an important role in transporting minerals, nutrients, and also heavy metals.[13] This pattern was also observed in *Sedum alfredii*, in which Zn was distributed mainly along the xylem.[14] The accumulation of Cr in *Gynura pseudochina* treated with Cr(III) was also located in the stem cortex and vascular bundle. Distribution, translocation and accumulation of Cr in *G. pseudochina* primarily depended on the oxidation state of Cr and on the plant tissue.[15] Studies have found that roots of *Typha latifolia* and *T. angustifolia* can accumulate Pb and Cr mainly in the epidermis.[16-18] Therefore, Cr in *T. angustifolia* of this study may perhaps still be in the upwards translocation and sequestration process because it was found mainly in the vascular bundle.

EtOAcThe detection of Cr in shoots indicate that Cr was transported from root to shoot. The specific tissue in which Cr was accumulated could not be determined based on Figure 4 and cross section observations are required to confirm it. There is a possibility that Cr is accumulated in tissues that are not directly involved in photosynthesis, in view of the fact that Cr can be toxic to the plant at even low concentrations. Therefore, Cr is expected to accumulate in leaf veins, to minimize damage to photosynthetic active tissue (mesophyll). The elements cadmium (Cd), cerium (Ce), copper (Cu), lanthanium (La), manganese (Mn), and zinc (Zn) are also potentially harmful to plants at high concentrations, and have been found to accumulate in the leaf veins of *Helianthus annuus.*[19] Not all of hyperaccumulator plants accumulate toxi-

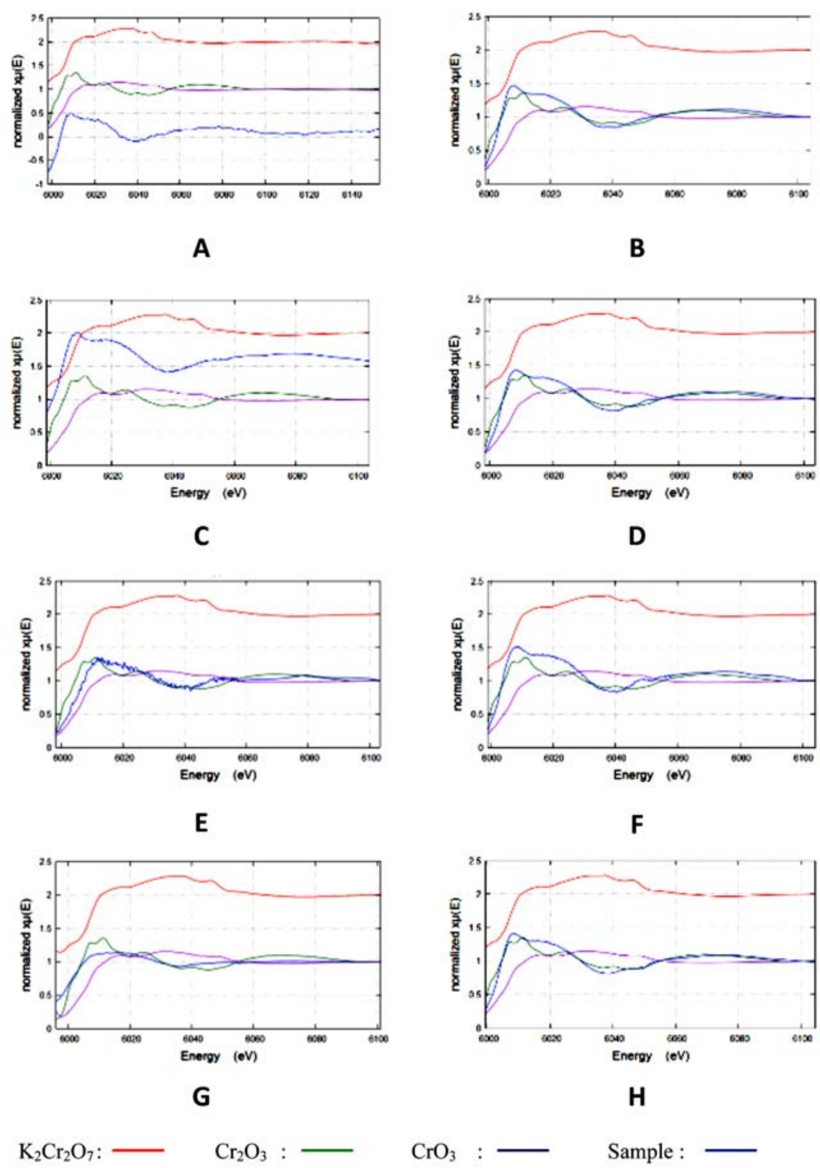

Figure 3. Cr K-edge XANES spectra of *T. angustifolia* shoot treated with 1, 5, 10, and 20 ppm Cr (A, C, E, and G) and root treated with 1, 5, 10, and 20 ppm Cr (B, D, F, and H).

cants in veins, *S. alfredii* can accumulate Zn not only in its epidermis or mesophyll but also in the center part of its leaf vein.[15]

EtOAcThe efficiency of heavy metal translocation from root to shoot is affected by various processes such as symplast uptaking by root, root sequestration, xylem loading and xylem unloading, as well as heavy metals uptaking by foliar cells. Researchers have found that increased xylem loading is the main factor affecting absorption and translocation of heavy metal from root to shoot; as shown in increasing uptake of Zn and Cd by *S. alfredii*, *Solanum melongena*, and *Solanum torvum.*[14,20] The xylem loading capacity of heavy metal hyperaccumulator plants is higher than in non-hyperaccumulator plants.[14,20] Hyperaccumulator plants also have special mechanisms to tolerate high concentration of heavy metals in tissue by using low-molecular-weight ligands, small metal-binding proteins such as histidine, organic acids, phytochelatine, metalothionine in the sequestration, transport, and accumulation of heavy metals.[21]

EtOAcCr(III) was the Cr species found to be accumulated in root and shoot of *T. angustifolia* in this study. Other researchers have found similar results in which Cr(III) was detected and accumulated in the root, stem and leaf of *Parkinsonia acculeta*, *Convolvulus arvensis* and *Medicago truncatula.*[22,23] Results from this study suggest that Cr(VI) may be reduced to Cr(III) in the root zone (rhizosphere). This zone creates a microenvironment for microorganisms; additionally, it has been found that roots release exudates that have the ability to reduce Cr(VI) to Cr(III) by adsorption. Biological activity in the rhizosphere and the availability of root exudates will affect heavy metal bioavailability.[24] Among the microbial communities found in the root zone are Fe-oxidizing bacteria. These bacteria can form iron oxide plaques around wetland plant roots. Another study found that Cr can bind with ferro (Fe) and Mn by forming a complex bond then become reduced to its trivalent form.[25] Plant species have different mechanisms to reduce Cr(VI) to Cr(III) within the plant. In *Trifolium brachycalycium*, *Atriplex canescence G. pseudochina* and *S. alfredii*, Cr(VI) is reduced to Cr(V) then further reduced to Cr(III), or Cr(VI) is directly reduced to Cr(III).[14,15,26]

EtOAcHyperaccumulator plants may develop different mechanisms applicable in the phytoremediation process. In this study, *T. angustifolia* tolerated Cr(VI) by reducing it to Cr(III) in the rhizosphere. Absorption of Cr(III) occurred via roots then transported in the root to the xylem. Cr(III) may have been distributed via the symplastic system into the cytoplasm and accumulated in the vacuole. Cr(III) in an organic complex may be translocated to the shoot via the xylem apoplastic system. Therefore, results of this study suggest that *T. angustifolia* has the posssibility of developing phytoextraction mechanisms which are recognized in the phytoremediation process. Phytoextraction is the plant's ability to uptake contaminants and translocate them to aboveground parts of the plant.[27]

Conclusions

EtOAcResults of this study found that Cr in the form of Cr(III) was detected in almost all parts of *T. angustifolia* root and shoot. Overall, the patterns of Cr distribution and speciation observed suggest that *T. angustifolia* in this study did not have a mechanism to reduce Cr(VI) to become Cr(III) inside the plants, but *T. angustifolia* has the posssibility to develop phytoextraction mechanism by translocating Cr(III) to above parts of the plant.

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