RESEARCH ARTICLE

Distribution of phenolic compounds in rice seedlings under Cr exposure

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Abstract: Responses of phenolic compounds were hydroponically investigated in rice seedlings (*Oryza sativa* L. cv. XZX 45) treated with either Cr(III) or Cr(VI). Results indicated that rice seedlings are able to effectively sequester both species of Cr. Majority of Cr recovered in plant materials was accumulated in roots rather than shoots. Accumulation of total soluble phenolics, flavonoids and lignin in plant materials was quite evident due to Cr exposure, but displaying different responses between the two species of Cr. Distribution of total soluble phenolics and flavonoids was more at shoots, especially at younger segments of shoots, and less at roots, whereas the lignin content was detected more at the younger parts of shoots and less towards the root tips. It is suggestive from the current investigation that both Cr species caused production and accumulation of these secondary metabolites in rice seedlings.
Keywords: chromium, phenolic, flavonoids, lignin, *Oryza sativa*

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1. Introduction

Chromium (Cr) widely distributes in the nature environment. Usually, trivalent and hexavalent chromium are considered to be the two most stable forms in the family of Cr. It has been reported that Cr(VI) is more water-soluble than Cr(III) and both chemical species are easily taken up by plants (Vajpayee et al., 2000). The former is classified as one of major toxic and mutagenic contaminants for living organisms. In some cases, the concentration of Cr has been detected up to several thousand mg/kg (Reeves and Baker, 2000). Cumulative domestic Cr slag in mainland China was estimated to be 6.0 million tons in 2005 (Jiang et al., 2013). Wastewater from leathery industry containing higher concentration of Cr is another major contaminated source to the receiving water, even if the maximum national allowable limit for total Cr (1.5 mg/L) and Cr(VI) (0.5 mg/L) in the effluent was recommended (Wang et al., 2007). In fact, the annual wastewater from tanning industries was approximately 180 million tons in 2005 and total Cr content was 2000-4000 mg/L (Wang et al., 2007).

Secondary metabolites are a group of small organic compounds naturally generated by plants. Their formation, distribution and function are highly dependent on species, organs and tissues of plants (Kliebenstein, 2004). Although involvement of secondary metabolites in the process of plant growth and development is not mandatory, they affect and/or change the relationship between plants and the environment (Yang et al., 2012). In most cases, plants are able to produce a large number of secondary metabolites in order to adapt to a variety of environmental stresses (Bartwal et al., 2012). Indeed, phenolic compounds, including flavonoids, lignin, and anthocyanins, are the most common species in the family of secondary metabolites. Being one of most important non-enzymatic antioxidants, it can chelate metal ions and serve as electron donors for guaiacol type peroxidase (Rice-Evans et al., 1996; Sgherri et al., 2003). It is evident that accumulation of phenolic compounds in plants can be induced by UV radiation, low nutrients, low temperature, heavy metals and pathogen attack (Yamasaki and Heshiki, 1995). For instance, stimulation of phenolic compounds has been detected in wheat exposed to Ni and Al (Winkel-Shirley, 2002). Although there are abundant literatures regarding phytotoxicity of Cr, no study is available concerning phenolic compounds in rice seedlings during Cr exposure. This work was aimed to investigate responses of phenolic compounds (total soluble phenolics, flavonoids and lignin) to different Cr species in rice seedlings, with emphasis on accumulation and distribution in different parts of young rice seedlings.

2. Materials and Methods

2.1. Plant Materials and Exposure Regime

Preparation of plant materials used was identical to our previous work (Yu and Feng, 2016). Seeds of rice (*Oryza sativa* L. cv. XZX 45) were planted in sand at 25°C and irrigated with a modified ISO 8692 nutrient solution (2823.9 μM

KNO_3, 59.0 μM $MgCl_2 \cdot 6H_2O$, 122.4 μM $CaCl_2 \cdot 2Hl_2O$, 60.9 μM $MgSO_4 \cdot 7H_2O$, 246.0 μM KH_2PO_4, 10.0 μM Fe-EDTA, 2992.1 nM H_3BO_3, 2097.0 nM $MnCl_2 \cdot 4H_2O$, 22.0 nM $ZnCl_2$, 6.3 nM $CoCl_2 \cdot 6H_2O$, 0.1 nM $CuCl_2 \cdot 2H_2O$ and 28.9 nM $NaMoOl_2 \cdot 2H_2O$). After 15 d of growth, young seedlings collected were incubated in a pre-treated solution containing 1 mM $CaCl_2$ + 2 mM MES-Tris buffer (pH 6.0) for 4 h to remove the ions from the cell wall space (Ebbs et al., 2008). All pre-treated young rice seedlings were used for the subsequent experiments.

Ten pre-treated seedlings with similar height and weight were exposed to 50 mL Cr-spiked solution containing different doses of Cr(VI) or Cr(III). Seven different concentrations of Cr(III) (0, 3.6, 7.2, 14.4, 21.6, 28.8 and 43.2 mg Cr/L) were prepared by adding the required aliquots of stock solution of chromium nitrate ($Cr(NO_3)_3 \cdot 9H_2O$) to the modified ISO 8692 nutrient solution. For the Cr(VI) treatments amended with potassium chromate (K_2CrO_4), seven different concentrations (0, 1.2, 2.4, 4.8, 7.2, 9.6, and 14.4 mg Cr/L) were applied. For each treatment concentration, four replicates were prepared. Exposure periods of all treatments were 3 days.

2.2. Measurement of Phenolic Compounds

Extraction procedure of phenolic compounds in plant materials was similar to previous studies (Kováčik et al., 2009; Li et al., 2008; Rodrigues et al., 2005) with slight modification. After exposing to Cr(VI) or Cr(III) for 3 days, rice seedlings were rinsed with distilled water and divided into roots (upper and lower segments) and shoots (upper and lower segments) (Figure 1). Plant materials (0.3 g fresh weight) were precisely weighted and homogenized on ice bath in 5 mL of 80% methanol. Then, the homogenate was centrifuged at 10,000×g for 5 min at 4°C. The supernatant was used for measurements of total soluble phenolics and flavonoids. The residue was also collected for determination of lignin content.

Assay of total soluble phenolics

Soluble phenolics were determined by the Folin-Ciocalteu method (Kováčik et al., 2009; Li et al., 2008) with slight modification. Briefly, 500 μL of supernatant was mixed with 300 μL of 1 N Folin and Ciocalteu's Phenol reagent. The mixture in the tube was placed on a rotating tube holder at 25°C for 5 min. And then, the solution was reacted with 400 μL of 1 M Na_2CO_3 and kept at 25°C for 10 min. Finally, the solution was made up to 5.0 mL with deionized water and allowed to stand for 1 h at 25°C. In order to avoid photo-oxidation, all tubes were covered with aluminum foil. The absorbance was measured at 725 nm. The blank contained 500 μL 80% methanol plus Na_2CO_3 and the Folin and Ciocalteu reagent. The content of total soluble phenolics was expressed in μg/g FW, based on the standard curve of gallic acid.

Assay of total flavonoids

The reconstituted fraction in methanol (1.0 mL supernatant) was mixed with 2.0 mL 80% methanol and 0.5 mL 5% $NaNO_2$ (w/v). After 5 min, 0.5 mL 10% $Al(NO_3)_3$ (w/v) was added to the mixture. The mixture was left standing for 6 min. 2.0 mL 4% NaOH was added and allowed to stand for 15 min. The absorbance was measured at 510 nm and calculation was based on the standard curve of rutin (Jia et al., 1999). The blank contained 1.0 mL 80% methanol plus other regents used.

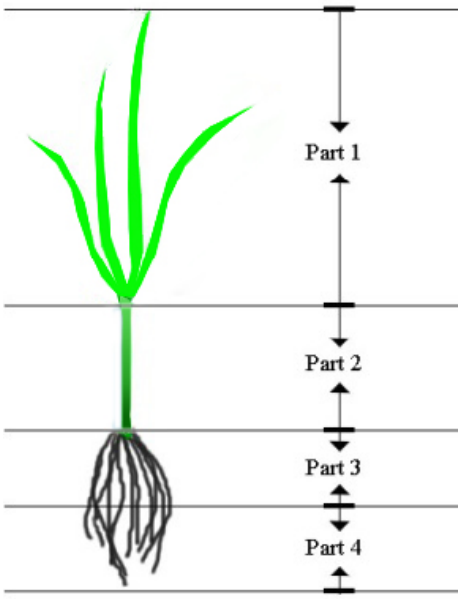


Figure 1. Graphic description of segments from rice seedlings used for analysis of phenolic compounds.

Assay of lignin

The resulting pellet obtained after extraction of total soluble phenolics was mixed with 5.0 mL deionized water and centrifuged at 10,000×g for 5 min at 4°C. The residue was collected and left to dry at 65°C overnight. A 5.0 mL of a 1:10 solution of thioglycolic acid and 2 M HCl were added to the dried residue. The mixture in the tube was homogenized on a rotating tube holder and then incubated in water bath at 100°C for 5 h. After cooling with ice bath to room temperature, the mixture was centrifuged at 10,000×g for 10 min at 4°C. The residue was collected and mixed with deionized water. After centrifuging at 10,000×g for 10 min, the residue pellet was collected again and resuspended with 3.0 mL 0.5 M NaOH. The mixture was placed on a rotating tube holder at room temperature overnight. The mixture was centrifuged at 10,000×g for 10 min. The supernatant was then transferred to a new tube. And then, 400 μL concentrated HCl was added and kept at dark for 4 h. Following centrifugation at 10,000×g for 10 min, the supernatant was discarded and the orange-brown precipitate was dissolved in 4.0 mL 0.5 M NaOH. The absorbance was measured at 280 nm and calculation was based on the standard curve of lignin alkali (Rodrigues et al., 2005).

2.3. Determination of Cr in Rice Seedlings

After exposure, seedlings collected were rinsed with deionized water and divided into roots and shoots. The remaining procedure was identical to our previous study (Yu et al., 2016). Plant materials were dried at 90°C for 48 h

and mixed with 8 mL of 4:1 HNO_3-$HClO_3$ solution for overnight. Then, samples were placed in a digestion block and heated for 2 h at 200°C until the digested liquid was clear. Then, the cooled residue was transferred into 50 mL glass flasks and added 0.2 mL 1% HNO_3. Finally, the solution was made up to 50 mL with deionized water. The concentration of total Cr in different parts of plant materials from different Cr treatments was analyzed by ICP-AES.

2.4. Statistical Analyses

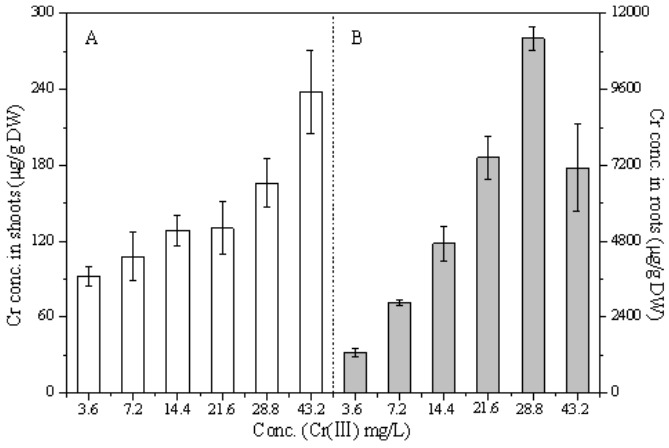
Analysis of variance (ANOVA) and Tukey's multiple range test was used to determine the statistical significance at 0.01 or 0.05 level between plant performances (Zar, 1999).

3. Results

3.1. Cr Accumulation in Plant Tissues

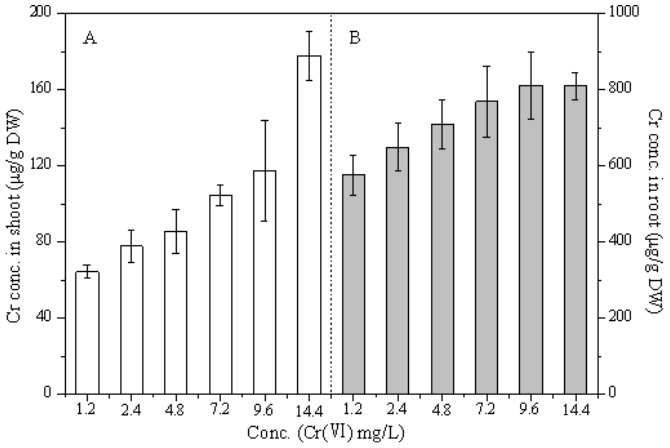
Figure 2 depicts the concentration (μg/g DW) of total Cr in roots of shoots of rice seedlings exposed to Cr(III) after 3 days exposure. Total Cr in roots was always significantly higher than that in shoots of rice plants at all treatment groups. Also, the Cr content in shoots showed a positive linear response to Cr(III) application (Figure 2A). However, Cr accumulation in roots responded biphasically to Cr(III) treatments, at which total Cr was linearly increased at 3.6-28.8 mg Cr/L, but decreased at 43.2 mg Cr/L (Figure 2B).

Compared with Cr(III) treatment, a similar distribution pattern of total Cr in plant materials was observed in Cr(VI) treatment, in which roots accumulated more Cr (Figure 3B) than shoots (Figure 3A). It is interesting to note that rice seedlings showed a greater capacity of sequestering Cr(III) than Cr(VI) within 3 days exposure. For instance, the rice seedling shoots accumulated 2863.43 μg/g DW when grown in the presence of Cr(III) at 7.2 mg Cr/L, while the roots accumulated only 768.72 μg/g DW at the Cr(VI) treatment (7.2 mg Cr/L).



Note: Values are mean of four replicates. Vertical lines represent standard deviation.

Figure 2. Accumulation of Cr (μg/g DW) in roots and shoots of rice seedlings exposed to Cr(III).



Note: Values are mean of four replicates. Vertical lines represent standard deviation.

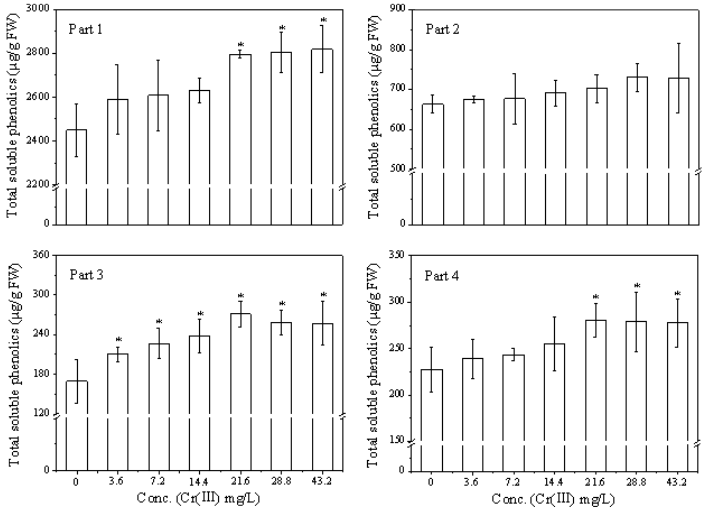
Figure 3. Accumulation of Cr (μg/g DW) in roots and shoots of rice seedlings exposed to Cr(VI).

3.2. Distribution of Total Soluble Phenolic in Plant Tissues

Figure 4 displayed the content of total soluble phenolics (μg/g FW) in different parts of roots and shoots of rice seedling. After exposure to Cr(III) for 3 days, an increase in total soluble phenolics was detected in both segments in shoots of rice seedlings. Slight increase was observed at Cr(III) treatments at less than or equal to 14.4 mg Cr/L ($P>0.05$) compared with non-treated rice seedling, whereas significant increase in total soluble phenolics was detected at 21.6 mg Cr/L or higher concentrations ($P<0.05$) in the upper parts of shoots. However, changes of total soluble phenolics in the low parts of shoots were marginal (Mean: 695.23 μg/g FW, SD: 26.2, No: 7), compared with non-treated rice seedlings ($P>0.05$). We also noted that the upper parts of shoots had significantly higher amounts of total soluble phenolics than the lower parts of shoots in both control and treatments ($P<0.05$).

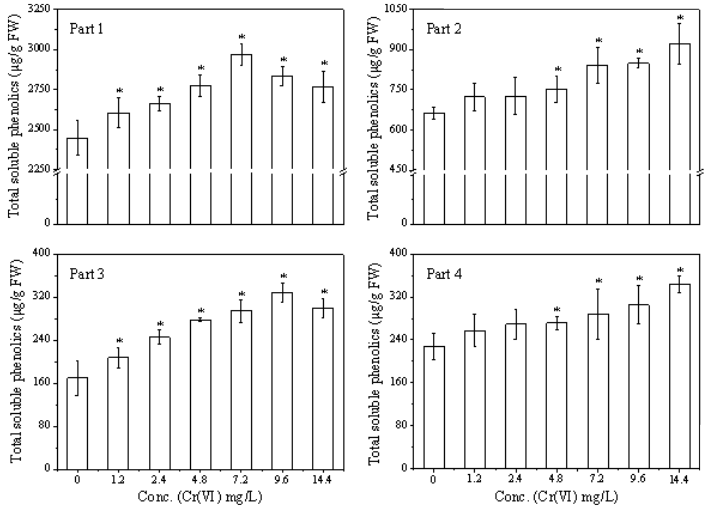
The total soluble phenolics in the upper parts of roots showed a remarkable response to Cr(III) application (Figure 4) when compared to control ($P<0.05$). In root tips (Part 4), significant increase was only detected at 21.6 mg Cr/L or higher concentrations ($P<0.05$), compared with non-treated rice seedlings. It is interesting to notice that shoots always had significant higher content than roots ($P<0.05$).

Significant increases of total soluble phenolics were detected in upper parts of shoots at all Cr(VI) treatments (Figure 5), compared with control ($P<0.05$). However, significant increases in total soluble phenolics were detected at 4.8 mg Cr/L or higher concentrations ($P<0.05$) in the lower parts of shoots ($P<0.05$). Similar responsive patterns were also observed in two segments of roots of rice seedlings after exposure to Cr(VI) for 3 days. It is noted that the distribution of total soluble phenolics in shoots was heterogeneous, while the distribution in roots was quite homogenous.



Note: Values are mean of four replicates. Vertical lines represent standard deviation. Asterisk refers to the significant difference between Cr-treated rice seedlings and control ($P<0.05$).

Figure 4. Measured total soluble phenolics content (μg/g FW) in roots and shoots of rice seedlings exposed to Cr(III).



Note: Values are mean of four replicates. Vertical lines represent standard deviation. Asterisk refers to the significant difference between Cr-treated rice seedlings and control ($P<0.05$).

Figure 5. Measured total soluble phenolics content (μg/g FW) in roots and shoots of rice seedlings exposed to Cr(VI).

3.3. Distribution of Total Flavonoids in Plant Tissues

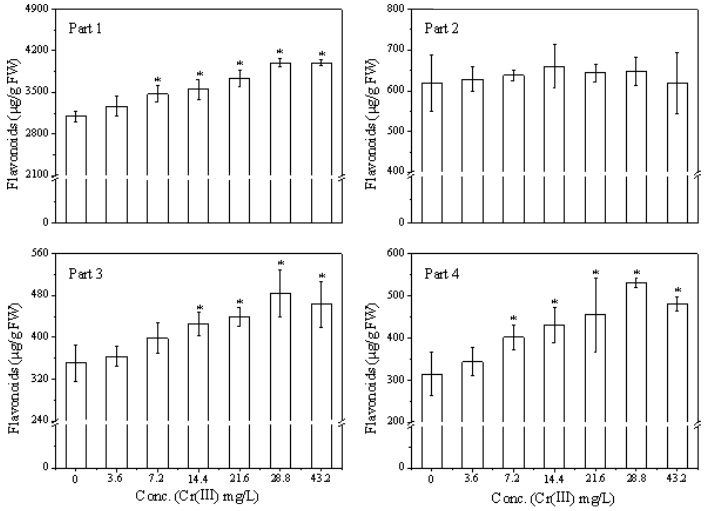
Responses of total flavonoids in different segments of rice seedlings exposed to Cr(III) were showed in Figure 6. At the treatment of 7.2-43.2 mg/L Cr(III), flavonoids content in upper parts of shoots was significantly increased ($P<0.05$), compared with control, while the changes of flavonoids content in lower parts of shoots were not significant (P>0.05) (Mean: 636.36 (μg/g FW, SD: 15.33, No: 7). In upper parts of roots, significant increase was only detected at 14.4 mg Cr/L or higher concentrations ($P<0.05$), compared with non-treated rice seedlings. At the treatment of 7.2-43.2 mg/L Cr(III), flavonoids content in root tips (Part 4) was significantly increased ($P<0.05$).

Significant increases in total flavonoids were detected in upper parts of shoots at all Cr(VI) treatments (Figure 7), compared with control ($P<0.05$), while the changes of flavonoids content in lower parts of shoots were not significant $P>0.05$) (Mean: 634.29 μg/g FW, SD: 13.35, No: 7). Remarkable increases in total flavonoids were observed in both segments of roots under the higher level of Cr(VI) (≥4.8 mg Cr/L).

3.4. Distribution of Lignin in Plant Tissues

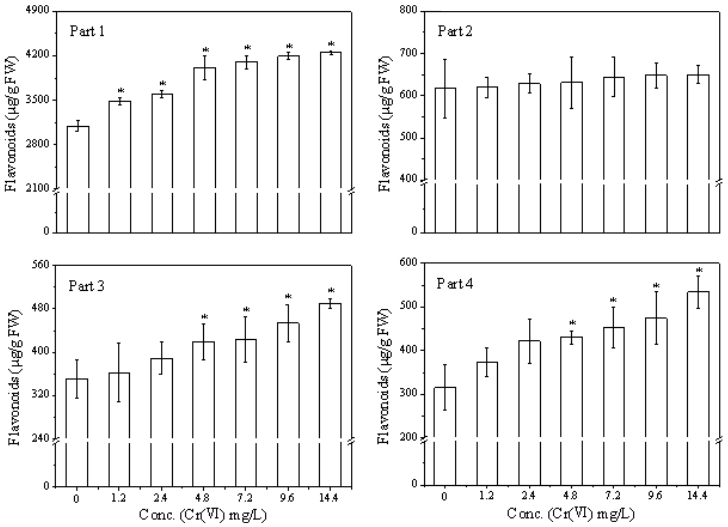
Changes of lignin content in different parts of plant materials of rice seedlings exposed to Cr(III) are given in Figure 8. Significant increases were observed in upper parts of shoots from all treatments compared with control ($P<0.05$), however the difference between six treatments was marginal (Mean: 11.91 mg/g FW, SD: 0.28, No: 6). A similar pattern was also detected in lower parts of shoots.

When exposed to Cr(VI), changes of lignin content in both segments of roots were identical, in which linear increases in lignin content were observed with an increase of



Note: Values are mean of four replicates. Vertical lines represent standard deviation. Asterisk refers to the significant difference between Cr-treated rice seedlings and control ($P<0.05$).

Figure 6. Measured total flavonoids content (μg/g FW) in roots and shoots of rice seedlings exposed to Cr(III).



Note: Values are mean of four replicates. Vertical lines represent standard deviation. Asterisk refers to the significant difference between Cr-treated rice seedlings and control ($P<0.05$).

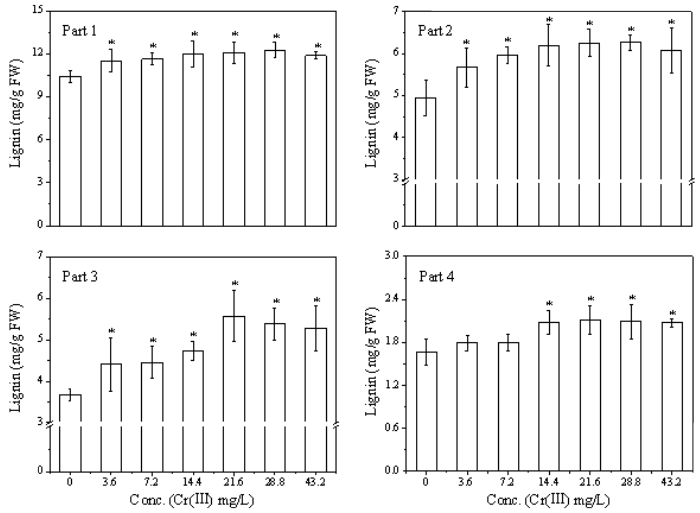
Figure 7. Measured total flavonoids content (μg/g FW) in roots and shoots of rice seedlings exposed to Cr(VI).

Cr(VI) concentrations (Figure 9). It is also noted that the lignin content was detected more at the upper parts of shoots (Part 1) and less towards the root tips (Part 4).

4. Discussion and Conclusion

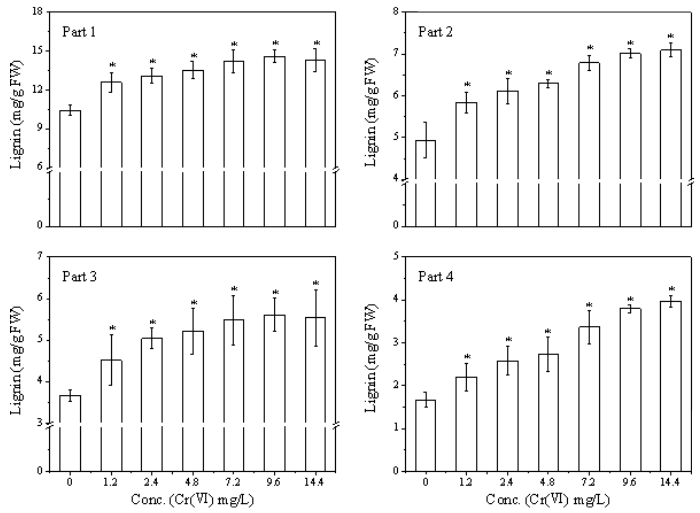
Although plants are able to take both species of Cr and more Cr was retained in roots rather shoots, less amounts of Cr was detected in shoots, especially in Cr(III) treatments, indicating that Cr(III) is less mobile than Cr(VI) within plant materials. In fact, more than 94.8% (SD: 2.89) of the total Cr(III) recovered was detected in roots, while 81.4% (SD: 4.67) of the total Cr was found in Cr(VI) treatments. This finding was similar to other studies (López-Luna et al., 2009), in which retention of Cr in roots of plants is one of the most important mechanisms for Cr(III). It is obvious that the translocation efficiency (TF) as the fraction that, after root uptake, is successfully translocated to upper parts of plants as defined by Meers et al. (2004). In this current study, TF values of Cr(VI) were estimated to be 10.1-18.1% for rice seedlings, whereas significant lower values of 1.7-6.7% were found in the Cr(VI) treatments. Such a difference is most likely due to the fact that Cr(III) may be transported in a passive way that Cr(VI) may be actively transported in plants by metabolic processes (Shanker et al., 2005; Yu et al., 2010).

In this current study, total soluble phenolics were mainly detected in upper parts of shoots (younger shoots), followed by older parts of shoots. The distribution of phenolics was quite homogenously in two segments of roots from young rice seedlings exposed to both species of Cr. In a previous work, relative abundance of total phenolics and flavonoids in the *Turnera subulata* was as



Note: Values are mean of four replicates. Vertical lines represent standard deviation. Asterisk refers to the significant difference between Cr-treated rice seedlings and control ($P<0.05$).

Figure 8. Measured lignin content (mg/g FW) in roots and shoots of rice seedlings exposed to Cr(III).



Note: Values are mean of four replicates. Vertical lines represent standard deviation. Asterisk refers to the significant difference between Cr-treated rice seedlings and control ($P<0.05$).

Figure 9. Measured lignin content (mg/g FW) in roots and shoots of rice seedlings exposed to Cr(VI).

follows: leaves>steams>flowers>roots>fruits (Chai and Wong, 2012). In order to reduce phytotoxicity of metal ions, a significant increase in total phenolics may be associated with some these soluble phenolics chelating metal ions. Indeed, soluble phenolics can directly chelate and/or bind with Cr, Pb and Hg with methanol extracts of rhizome polyphenols from Nympheae (Lavid et al., 2001). This chelating ability of phenolic compounds is possible related to the high nucleophilic character of the aromatic rings rather than to specific chelating groups within the molecule (Moran et al., 1997).

Flavonoids accumulation in both roots and shoots of rice seedlings exposed to either Cr(III) or Cr(VI) was detected in this study. Mechanisms of flavonoids generation may be due to the fact that it can help maintain membrane integrity by preventing the hydrophobic region of the biolayer from being accessed by the deleterious molecules (Bartwal et al., 2012). Also, the role of flavonoids was able to donate electrons or hydrogen atoms during scavenging ROS, such as superoxide, singlet oxygen, hydrogen peroxide, hydroxyl radical and peroxyl radical (Sakihama et al., 2000).

Significant increase in lignin content was observed in both roots and shoots of rice seedlings after exposing to both Cr species. Also, the lignin content was detected more at the upper parts of shoots (Part 1) and less towards the root tips (Part 4). Excess Al also caused lignin deposition in the cell wall of wheat, consequently higher accumulation of lignin in the cell wall resulted in growth inhibition, which is independent on the tolerance to Al (Sasaki et al., 1996). Also, Cr is able to induce the production of lignin in roots of *Phragmites australis* (Zagoskina et al., 2007). Being as the product of secondary cell wall, plants are able to produce more lignin to prevent heavy metals into the interior and protect the normal growth of pants. In fact, the covalent bonding

of functional groups induced polymerization, condensation, and complexation reactions, chiefly achieved at the cell wall. Finally, lignin was formed through polymerization of phenolic alcohols (Bartwal et al., 2012).

In summary, rice seedlings are able to effectively take up both Cr species, and mainly accumulate in roots rather than shoots. Production of total soluble phenolics, flavonoids and lignin in plant materials was quite evident in rice seedlings exposed to either Cr(III) or Cr(VI), but responses to both Cr species are different. The distribution of three secondary metabolites is chiefly located in shoots, especially in younger shoots.

Author Contributions

Fei-Fei Zhang performed the experiments and collected data. Xiao-Zhang Yu conceived the study, conducted data analysis and drafted the manuscript. All authors read and approved the final manuscript.

Conflict of Interest and Funding

The authors declare no conflict of interest. This work was financially supported by the research foundations from Guilin University of Technology (Grant No. GUTRC2011007) and The Guangxi Talent Highland for Hazardous Waste Disposal Industrialization.

References

Bartwal, A., Mall, R., Lohani, P., Guru, S.K. and Arora, S., 2012. Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. Journal of Plant Growth Regulation, 32(1), 216-232. https://doi.org/10.1007/s00344-012-9272-x.

Chai, T.T. and Wong, F.C., 2012. Whole-plant profiling of total phenolic and flavonoid contents, antioxidant capacity and nitric oxide scavenging capacity of Turnera subulata. Journal of Medicinal Plants Research, 6(9), 1730-1735.

Ebbs, S.D., Piccinin, R.C., Goodger, J.Q.D., Kolev, S.D., Woodrow, I.E. and Baker, A.J.M., 2008. Transport of ferrocyanide by two eucalypt species and sorghum. International Journal of Phytoremediation, 10(4), 343-357. https://doi.org/10.1080/15226510802096242.

Jia, S.Z., Tang M.C. and Wu J.M., 1999, The determination of flavonoide contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64(4),555-559. https://doi.org/10.1016/S0308-8146(98)00102-2.

Jiang, L.L., Li, R.W., Mao, Y.Q. and Zhou, M., 2013. Present processing technology and comprehensive utilization of chromium slag. Environmental Science & Technology, 36, 480-483.

Kliebenstein, D.J., 2004.Secondary metabolites and plant/environment interactions: a view through *Arabidopsis thaliana* tinged glasses. Plant, Cell & Environment, 27(6), 675-684.

Kováčik, J., Klejdus, B. and Bačkor, M., 2009. Phenolic metabolism of Matricaria chamomilla plants exposed to nickel. Journal of Plant Physiology, 166(13), 1460-1464.

Lavid, N., Schwartz, A., Yarden, O. and Tel-Or, E., 2001. The involvement of polyphenols and peroxidase activities in heavy-metal accumulation by epidermal glands of the waterlily (Nymphaeaceae). Planta, 212(3), 323-331. https://doi.org/10.1007/s004250000400.

Li, L., Shewry, P.R. and Ward, J.L., 2008. Phenolic acids in wheat varieties in the HEALTH GRAIN diversity screen. Journal of Agricultural and Food Chemistry, 56(21), 9732-9739. https://doi.org/10.1021/jf801069s.

López-Luna, .J, González-Chávez, M.C., Esparza-García, F.J., Rodriguez-Vázguez, R., 2009. Toxicity assessment of soil amended with tannery sludge, trivalent chromium and hexavalent chromium, using wheat, oat and sorghum plants. Journal of Hazardous Materials, 163(2-3), 829-834.

Meers, E., Hopgood, M., Lesage, E., Vervaeke, P., Tack, F.M.G. and Verloo, M.G., 2004. Enhanced phytoextraction: in search of EDTA alternatives. International Journal of Phytoremediation, 6(2), 95-109. https://doi.org/10.1080/16226510490454777.

Moran, J.F., Klucas, R.V., Grayer, R.J., Abian, J. and Becana, M., 1997. Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: prooxidant and antioxidant properties. Free Radical Biology and Medicine, 22(5), 861-870. https://doi.org/10.1016/S0891-5849(96)00426-1.

Reeves, R.D and Baker, A.J.(2000) Metal accumulating plants. pp.193-229. *In*: Raskin I and Ensley BD (eds), Phytoremediation of Toxic Metals Using Plants to Clean up the Environment. John Wiley & Sons Inc, New York.

Rice-Evans, C.A., Miller, N.J. and Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20(7), 933-956. https://doi.org/10.1016/0891-5849(95)02227-9.

Rodrigues, F.Á., Jurick, W.M., Datnoff, L.E., Jones, J.B. and Rollins, J.A., 2005. Silicon influences cytological and molecular events in compatible and incompatible rice-Magnaporthe grisea interactions. Physiological and Molecular Plant Pathology, 66(4), 144-159. https://doi.org/10.1016/j.pmpp.2005.06.002.

Sakihama, Y., Mano, J.I., Sano, S., Asada, K. and Yamasaki, H., 2000. Reduction of phenoxyl radicals mediated by monodehydroascorbate reductase. Biochemical and Biophysical Research Communications, 279(3), 949-954. https://doi.org/10.1006/bbrc.2000.4053.

Sasaki, M., Yamamoto, Y. and Matsumoto, H., 1996. Lignin deposition induced by aluminum in wheat (*Triticum aestivum*) roots. Physiologia Plantarum, 96(2), 193-198. https://doi.org/10.1111/j.1399-3054.1996.tb00201.x.

Sgherri, C., Cosi, E. and Navari-Izzo, F., 2003. Phenols and antioxidative status of *Raphanus sativus* grown in copper excess. Physiologia Plantarum, 118(1), 21-28. https://doi.org/10.1034/j.1399-3054.2003.00068.x.

Shanker, A.K., Cervantes, C., Loza-Tavera, H. and Avudainayagam, S., 2005. Chromium toxicity in plants. Environment International, 31(5), 739-753. https://doi.org/10.1016/j.envint.2005.02.003.

Vajpayee, P., Tripathi, R.D., Rai, U.N., Ali, M.B. and Singh, S.N., 2000. Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L. Chemosphere, 41(7), 1075-1082. https://doi.org/10.1016/S0045-6535(99)00426-9.

Wang J.Y., Su H.J. and Tan T.W., 2007. Study on reuse and treatment of tannery chrome effluents. Chinese Journal of Environmental Engineering, 1(1), 23-27.

Winkel-Shirley, B., 2002. Biosynthesis of flavonoids and effects of stress. Current Opinion in Plant Biology, 5(3), 218-223. https://doi.org/10.1016/S1369-5266(02)00256-X.

Yamasaki, H., Heshiki, R. and Ikehara, N., 1995. Leaf-goldenning induced by high light in Ficus microcarpa L. f., a trop-

ical fig. Journal of Plant Research, 108(2), 171-180. https://doi.org/10.1007/BF02344341.

Yang, C.Q., Fang, X., Wu, X.M., Mao, Y.B., Wang, L.J. and Chen, X.Y., 2012. Transcriptional regulation of plant secondary metabolism. Journal of Integrative Plant Biology, 54(10), 703-712. https://doi.org/10.1111/j.1744-7909.2012.01161.x.

Yu, X.Z., Peng, X.Y. and Xing, L.Q., 2010. Effect of temperature on phytoextraction of hexavalent and trivalent chromium by hybrid willows. Ecotoxicology, 19(1), 61-68. https://doi.org/10.1007/s10646-009-0386-2.

Yu, X.Z. and Feng, X.H., 2016. Effects of trivalent chromium on biomass growth, water use efficiency and distribution of nutrient elements in rice seedlings. Applied Environmental Biotechnology, 1(1), 64-70. https://doi.org/10.18063/AEB.2016.01.005.

Yu, X.Z., Zhang, F.F. and Liu, W., 2016. Chromium-induced depression of ^{15}N content and nitrate reductase activity in rice seedlings. International Journal of Environmental Science and Technology, 14(1), 29-36. https://doi.org/10.1007/s13762-016-1130-0.

Zagoskina, N.V., Goncharuk, E.A. and Alyavina, A.K., 2007. Effect of cadmium on the phenolic compounds formation in the callus cultures derived from various organs of the tea plant. Russian Journal of Plant Physiology, 54(2), 237-243. https://doi.org/10.1134/S1021443707020124.

Zar J.H., 1999. Biostatistical Analysis (4rd ed). New Jersey: Prentice Hall. pp. 231-261.