RESEARCH ARTICLE

Chemical forms of chromium in rice plants: does this fraction determine its phytotoxicity?

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Abstract: Chemical forms of chromium (Cr) in rice seedlings (*Oryza sativa* L. cv. BX139) exposed to either potassium chromate Cr(VI) or chromium nitrate Cr(III) were clarified using a hydroponic study. Seven chemical fractions of Cr in different rice tissues were extracted using a sequential extraction method. Results indicated that exposures to both Cr valents resulted in significant accumulation of Cr in rice tissues and Cr(III) was more bioavailable for rice seedlings than Cr(VI). However, Cr chemical forms were inconsistent in both plant materials (root/shoots) as well as in two different Cr variants. Although both Cr variants caused dose-dependent inhibition on relative growth rates of rice seedlings, different inhibition mechanisms most likely exited using a partial correlation analysis. Both fractions of Cr in cell wall and in intracellular location in roots significantly inhibited the relative growth rates of rice seedlings exposed to Cr(VI), while inhibition of the relative growth rate of rice seedlings exposed to Cr(III) was largely stemmed from Cr partition in intracellular fraction in shoots.
Keywords: chromium; cell wall; intracellular fraction; relative growth rate; rice seedlings

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1 Introduction

Diverse anthropogenic activities have caused high-volume release of heavy metals into the environment. Due to their non-biodegradability, heavy metals are able to couple with the tendency for bio-enrichment into crop plants, which pose a potential threat to human health (Sharma and Dietz, 2008). It was evident that metal tolerance and detoxification are two survival strategies for plants to cope with heavy metal stresses (Sousa et al., 2008).

Compartmentalization of heavy metals in cell wall deposition and vacuolar compartmentation has been regarded as two significant processes involved in heavy metal tolerance and detoxification (Cobbett, 2003; Weng et al., 2012; Lai, 2015; Aryal et al., 2016; Yu et al., 2018). Chromium (Cr) is one of the most frequently detected heavy metals in the environment either from natural input or from anthropogenic activities. It is known that Cr(VI) and Cr(III) are the two most common and stable chemical species in the family of Cr. However, their biological effects are highly dependent on its individual chemical properties, occurrence and behavior (Yu et al., 2017). In fact, there are two typical methods to elucidate metal toxicity based on cellular metal fates: a chemical form approach and the subcellular partitioning model (Li et al., 2014). In our previous work, a remarkable difference in subcellular distribution of Cr in rice tissues was observed between two Cr treatments (Yu et al., 2018). Chemical form method is able to clarify metal fates within plant tissues, especially emphasizing the complex forms and mobility of metal ions, using a sequential extraction procedure (Wu et al., 2005; Sousa et al., 2008; Wang et al., 2008; Zhang et al., 2014). It has been reported that metals binding to pectates, phosphates, oxalates and residuals are less toxic to terrestrial plants, suggesting that chemical forms could be one of the most important heavy metal detoxification mechanisms (Wang et al., 2008; Fu et al., 2011; Weng et al., 2012; Xu and Wang, 2013). This current study aims to investigate chemical forms of Cr in rice seedlings exposed to either Cr(VI) or Cr(III), with the objective to provide useful information to clarify which fraction from either cell wall or intracellular location carried more weight in phytotoxicity of Cr to rice seedlings.

2 Materials and Methods

2.1 Rice seedlings

Rice (*Oryza sativa* L. cv. BX139) seedlings were prepared as previously described (Yu et al., 2017). Seeds of rice were planted in sandy soils under laboratory condition at 25°C and irrigated with 25%-strength Hoagland's nutrient solution. After 20 days of growth, seedlings were collected and rinsed with distilled water. All pre-incubated young seedlings with ions cleaning buffer (Ebbs et al., 2008) were used for the subsequent experiments. Tests were conducted in a plant growth chamber with constant temperature of 25± 0.5 °C and a relative humidity of 60±2 % under continuous artificial light. Four independent biological replicates were performed for each Cr treatment.

2.2 Exposure Regime

Ten pre-treated young rice seedlings with similar height and weight were exposed to 50 mL Cr-spiked solution containing different doses of Cr(VI) and Cr(III). The initial concentrations of Cr in treatments spiked with Cr(III) (chromium nitrate) were 2.0, 4.0, 8.0, 16.0, 24.0, and 32.0 mg Cr/L, while the initial Cr concentrations in treatments amended with Cr(VI) (potassium chromate) were 1.0, 2.0, 4.0, 8.0, 12.0 and 16.0 mg Cr/L.

2.3 Relative Growth Rate

Fresh weight of rice seedlings was measured prior to application and then at the termination of exposure in order to determine the relative growth rate (%) (Yu and Zhang, 2017).

2.4 Extraction of Cr in Different Chemical Forms

Seven chemical forms of Cr were extracted following a sequential extraction procedure (Sousa et al., 2008; Wang et al., 2008; Zhang et al., 2014). After exposing to Cr(VI) or Cr(III) for 3 days, rice seedlings were rinsed with distilled water and divided into roots and shoots. Treated and non-treated plant tissues were precisely weighted and immediately ground. After that, different designated extractants were used. All extraction steps were agitated on a shaker at 150 rpm for 24 hours at room temperature. After each extraction step, the mixture was centrifuged at 5,000 × *g* for 10 min at 4°C and the supernatants were collected for Cr analysis in different forms. The content of total Cr in different fractions in plant materials of rice seedlings from both Cr treatments was determined by ICP-AES (Yu and Feng, 2016). (1) Ethanol fraction (F_E): using 80% ethanol to extract inorganic Cr, which included nitrate/nitrite, chloride and aminophenol Cr; (2) Water fraction (F_W): using deionized water to extract water-soluble Cr of organic complexes; (3) NaOH fraction (F_{NaOH}): using 0.5 M NaOH to extract Cr complexed with polissacaridic compounds; (4) NaCl fraction (F_{NaCl}): using 1 mg/L NaCl to extract Cr integrated with pectates and protein; (5) Acetic acid fraction (F_{HAC}): using 2% acetic acid to extract Cr-phosphate complexes; (6) HCl fraction (F_{HCl}): using 0.6 mg/L HCl to extract oxalate acid bound Cr; (7) Residue fraction (F_R): acid digestion of the plant residue was performed with $HNO_3/HClO_4$(4:1) (Yu and Feng, 2016).

2.5 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. The partial correlation was used to determine whether a relationship between two variables was due to a common correlation to a third variable, with the equation

$$r_{xy.z} = \frac{r_{xy} - r_{xz} \times r_{yz}}{\sqrt{(1 - r_{xz}^2) \times (1 - r_{yz}^2)}}$$

Where

$r_{xy.z}$ is the partial correlation coefficient between variables x and y under the assumption of a constant variable *z*, and r_{xy} is the bivariate Pearson correlation coefficient between variables *x* and *y* etc..

3 Results

3.1 Relative Growth Rate of Rice Seedlings

Relative growth rate of rice seedlings was used as the sensitive endpoint to determine phytotoxicity of Cr to both Cr variants. In both Cr treatments, relative growth rates of rice seedlings displayed a dose-dependent decrease to Cr exposure. Compared with non-treated seedlings, relative growth rate of rice seedlings exposed to Cr(VI) at 2.0 mg/L or higher concentrations significantly decreased ($P < 0.05$) (Figure 1a), while Cr(III) at 16.0 mg/L or higher concentrations caused remarkable inhibition on biomass growth ($P < 0.05$) (Figure 1b).

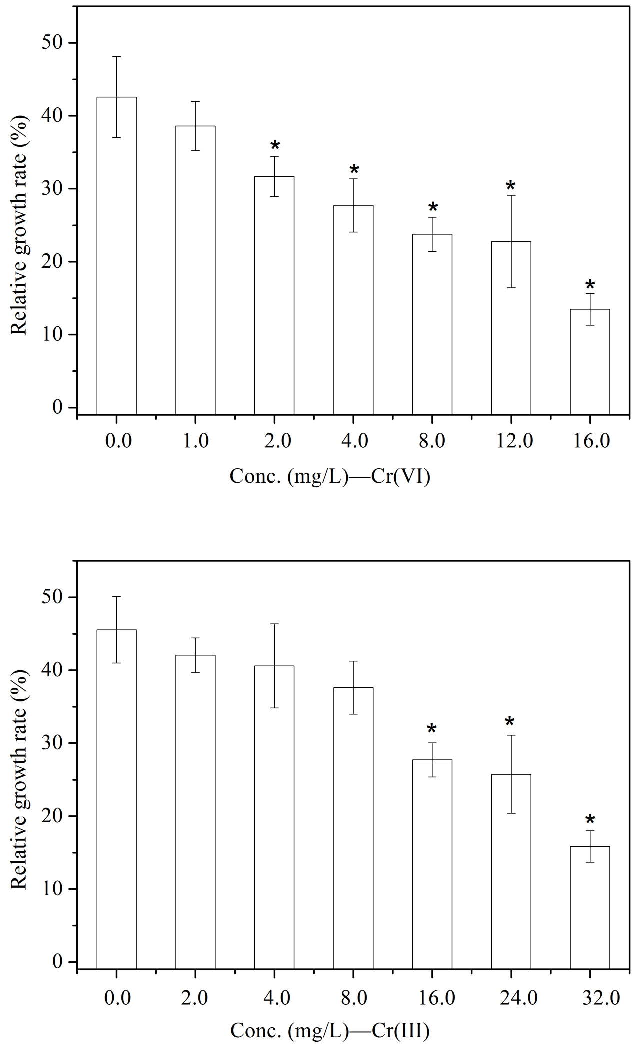

Figure 1. Measured relative growth rate (%) of rice seedlings exposed to Cr(VI) (Figure 1a) or Cr(III) (Figure 1b). The exposure period was 48 h. Values are mean of four independent biological replicates. Vertical lines represent standard deviation. Asterisk refers to the significant difference between Cr treatments and control ($p < 0.05$).

Table 1. Total Cr concentrations (μg/g FW) in different fractions from rice tissues exposed Cr(VI). Values are mean of four replicates. Numerical values in brackets represent SD.

Rice tissue	Fraction	Cr concentration (μg/g FW)					
		1.0	2.0	4.0	8.0	12.0	16.0
Root	F_E	7.22 (1.11)	11.02 (2.38)	11.92 (2.15)	23.24 (1.94)	23.68 (3.38)	24.34 (2.23)
	F_W	7.64 (0.8)	8.05 (0.97)	7.72 (1.8)	8.48 (0.96)	9.18 (1.14)	10.92 (1.28)
	F_{NaOH}	16.19 (1.48)	34.41 (3.92)	41.27 (4.28)	77.77 (7.72)	81.47 (7.07)	78.55 (7.45)
	F_{NaCl}	0.76 (0.06)	1.2 (0.13)	1.44 (0.41)	1.46 (0.08)	1.93 (0.09)	1.61 (0.21)
	F_{HAC}	0.37 (0.02)	0.18 (0.06)	0.21 (0.14)	1.14 (0.04)	1.17 (0.19)	1.66 (0.11)
	F_{HCl}	1.66 (0.3)	2.35 (0.6)	3.26 (1.01)	3.2 (0.25)	3.86 (2.17)	5.16 (1.17)
	F_R	10.81 (1.52)	9.8 (4.83)	9.02 (0.88)	11.83 (2.18)	13.09 (1.44)	14.67 (2.48)
	Total	44.65 (6.93)	67.01 (16.88)	74.84 (13.97)	127.12 (17.25)	134.38 (20.27)	136.91 (19.56)
Shoot	F_E	3.25 (0.59)	4.43 (0.41)	3.63 (0.78)	6.71 (1.66)	4.77 (1.87)	12.97 (1.59)
	F_W	0.92 (0.29)	1.43 (0.38)	2.31 (0.74)	6.16 (1.12)	5.73 (1.68)	7.37 (1.33)
	F_{NaOH}	0.8 (1.04)	2.07 (1.45)	3.19 (2.03)	4.58 (3.67)	8.69 (2.15)	13.09 (4.58)
	F_{NaCl}	0.49 (0.16)	0.54 (0.04)	0.58 (0.04)	0.82 (0.11)	0.95 (0.25)	1.06 (0.18)
	F_{HAC}	0.31 (0.14)	0.46 (0.18)	0.61 (0.13)	0.6 (0.22)	0.83 (0.52)	0.62 (0.23)
	F_{HCl}	0.06 (0.01)	0.17 (0.1)	0.29 (0.14)	1.35 (0.46)	1.4 (0.77)	1.72 (0.26)
	F_R	1.94 (0.28)	1.79 (0.19)	2.09 (0.28)	2.94 (0.27)	3.56 (1.26)	4.01 (1.45)
	Total	7.77 (3.28)	10.89 (3.60)	12.7 (5.42)	23.16 (9.84)	25.93 (11.14)	40.84 (12.60)

3.2 Cr distribution and Chemical Forms in Rice Seedlings Exposed to Cr(VI)

After a 3-day Cr(VI) exposure, total Cr in different parts of non-treated and treated rice seedlings was all measured. The background of Cr in rice tissues from non-treated seedlings was all below detection limit. Substantial differences in Cr distribution were observed between rice tissues after Cr(VI) exposure, in which the binding pools for Cr in rice seedlings was mainly located in roots rather than shoots. Indeed, almost 84% (Mean: 83.69%, SD: 3.35, No: 6) of Cr recovered in rice tissues were detected in roots of rice seedlings (Table 1).

In Cr(VI) treatments (Figure 2a), Cr chemical forms were variable between rice tissues. Most Cr in roots was in NaOH fraction (36.3-61.2%). It is interesting to note that Cr in NaOH fraction (%) in roots remained almost unchanged when exposed to Cr(VI) at 2.0 mg/L or higher concentrations. Cr partition (%) in ethanol fraction from all Cr(VI) treatments kept constantly (Mean: 17%, SD: 0.98). Cr in both water and residue fractions was negatively correlated with Cr(VI) concentration from 1.0 mg/L to 4.0 mg/L, while unchanged Cr fraction was observed at 8.0 mg/L or higher concentrations. Less than 5% was detected in NaCl and acetic acid and HCl fraction. Cr chemical forms in shoots were completely different compared with roots. Most Cr was detected in ethanol fraction. Generally, Cr partition (%) in water, NaOH and HCl fraction was positively correlated with Cr(VI) treatments in shoots, while negative correlations were observed in NaCl and acetic acid fraction.

3.3 Cr Distribution and Chemical Forms in Rice Seedlings Exposed to Cr(III)

In Cr(III) treatments, major accumulation of Cr in rice tissues was observed in roots rather shoots. In fact, more than 93% (Mean: 93.6%, SD: 4.16, No: 6) of Cr recovered in rice tissues were detected in roots of rice seedlings exposed to Cr(III) (Table 2).

In Cr(III) treatments (Figure 2b), most Cr in roots from Cr(III) treatments was detected in HCl (22.9-41.7%) and residue (33.8-39.6%) fraction, followed by NaCl fraction. The smallest (0.87-2.51%) was detected in acetic acid fraction in roots. In shoots, most Cr was in NaOH fraction when exposed to Cr(III) at 2.0 mg/L or less concentrations, while more Cr was detected in residue fraction with increasing Cr(III) concentrations.

Table 2. Total Cr concentrations (μg/g FW) in different fractions from rice tissues exposed Cr(VI). Values are mean of four replicates. Numerical values in brackets represent SD.

Rice tissue	Fraction	Cr concentration (μg/g FW)					
		2.0	4.0	8.0	16.0	24.0	32.0
Root	F_E	7.99 (3.23)	10.04 (0.9)	10.18 (1.65)	20.93 (3.56)	34.31 (4.08)	45.77 (4.98)
	F_W	10.03 (0.86)	9.67 (1.65)	10.85 (1.82)	45.02 (4.47)	59.33 (1.87)	81.07 (3.13)
	F_{NaOH}	6.15 (1.29)	6.32 (1.42)	6.53 (1.42)	15.85 (2.9)	18.84 (2.91)	32.14 (3.3)
	F_{NaCl}	20.56 (2.69)	37.12 (2.88)	41.55 (4.26)	126.71 (9.15)	161.25 (9.38)	222.71 (7.95)
	F_{HAC}	0.92 (0.1)	3.78 (1.1)	10.1 (2.44)	14.99 (2.68)	16.82 (1.55)	21.5 (1.78)
	F_{HCl}	24.18 (4.48)	50.22 (3.6)	164.11 (10.5)	289.33 (16.72)	519.49 (56.6)	682.27 (35.19)
	F_R	35.67 (3.69)	63.86 (4.02)	159.67 (33.69)	331.52 (57.25)	436.53 (43.08)	579.05 (36.32)
	Total	105.5 (21.50)	181.01 (19.31)	403.04 (69.17)	844.35 (119.95)	1246.57 (148.14)	1664.51 (114.86)
Shoot	F_E	0.45 (0.1)	1.18 (0.49)	1.17 (0.49)	2.08 (0.59)	2.04 (0.69)	3.33 (0.46)
	F_W	1.45 (0.16)	1.52 (0.27)	1.57 (0.31)	2.71 (0.86)	3.85 (0.66)	4.14 (1.55)
	F_{NaOH}	7.98 (2.3)	6.81 (1.2)	6.09 (0.88)	6.75 (0.77)	6.98 (2.13)	13.92 (1.31)
	F_{NaCl}	1.57 (0.43)	1.95 (0.51)	2.09 (0.51)	4.11 (1.77)	6.13 (1.62)	8.38 (3.01)
	F_{HAC}	0.35 (0.09)	0.65 (0.43)	1.22 (1.02)	2.36 (1.14)	4.9 (1.2)	4.08 (2.23)
	F_{HCl}	1.17 (1.31)	1.13 (0.48)	1.82 (0.6)	3.77 (1.17)	6.23 (1.39)	15.43 (2.5)
	F_R	3.71 (1.05)	5.05 (0.86)	6.36 (1.54)	9.17 (0.9)	13.27 (1.63)	17.75 (5.51)
	Total	16.68 (5.51)	18.29 (5.25)	20.32 (6.63)	30.95 (8.92)	43.4 (11.56)	67.03 (20.55)

4 Discussion and Conclusion

In this investigation, rice seedlings are able to sequester and transport both species of Cr. Roots are the major site for Cr accumulation from both Cr treatments. However, different rates in uptake and accumulation were detected between two Cr treatments. Indeed, Cr(III) was more bioavailable for rice seedlings than (VI); Cr accumulation in roots was more evident in Cr(III) treatments than Cr(VI). Additionally, we also noted that Cr(VI) is more mobile in plant materials than Cr(III), judged by the ratio of metal concentrations in shoots to roots, which is defined as translocation factor (TF). The TF value was 20.7% for rice seedlings exposed to Cr(VI), whereas 4.42% was observed in case of Cr(III) treatments. These results suggest that different channels of uptake and accumulation most likely existed between two Cr variants, and in vivo conversion of Cr(VI) into Cr(III) within plant tissues is unlikely to occur during transport. A similar conclusion was also reached by other studies (Yu et al., 2018).

It is evident that metal location in plant tissues can be separated into three sections: cell wall, proteic fraction and intracellular location (Sousa et al., 2008). Intracellular metals refer to the soluble metals in plant tissues, which are extracted by ethanol and deionized water, whereas cell wall fraction includes other parts extracted by NaOH, NaCl, Acetic acid, HCl, and $HNO_3/HClO_4$. The current used extraction method was unable to determine the proteins exact location in the cell (Sousa et al., 2008). In this work, Cr(VI) in intracellular location accounted for 27.2% (SD. 3.29, n=6) in roots, while majority of Cr(VI) accumulated was in cell wall fraction with a value of 72.8%. However, a different pattern of Cr(VI) distribution was observed in shoots, in which Cr(VI) location between cell wall and intracellular fraction was quite homogeneous. In Cr(III) treatments, more Cr was deposited in cell wall fraction in both roots and shoots, while only small fraction of Cr(III) was detected in intracellular location. It has been reported that compartmentalization is an effective strategy for plants to minimize phytotoxicity to heavy metals (Cobbett, 2003; Lai, 2015) since primary physiological function zones were chiefly located in cytosol and organelle rather than cell wall in plants. In fact, plant cell wall contains protein and polysaccharides, such as lignin and cellulose, which include ligands like carboxyl, aldehyde, and hydroxyl (Ren et al., 2014; Aryal et al., 2016). These substances acting as ligands are capable of binding metal ions and depositing in cell walls (Yu et al., 2017).

In this study, both Cr variants caused dose-dependent inhibition on relative growth rates of rice seedlings. Analysis of chemical forms of Cr showed that seven different fractions at

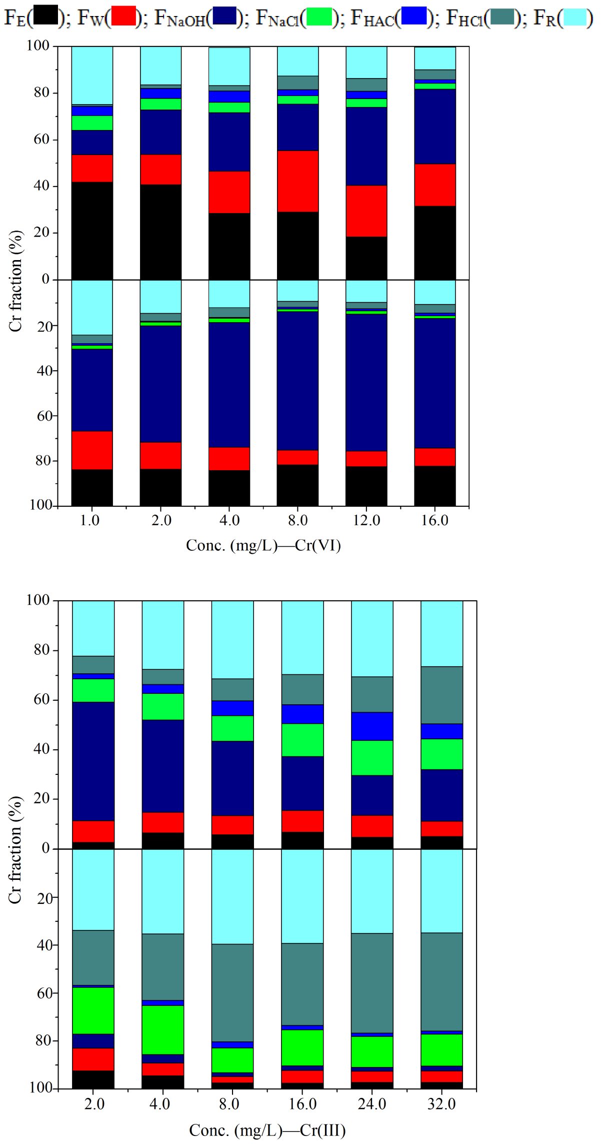

Figure 2. Proportion of different chemical form components of Cr in rice tissues exposed to Cr(VI)) (Figure 2a) or Cr(III) (Figure 2b). Upper part refers to the data from roots and lower part is from shoots.

different rates were detected in rice tissues, which is further categorized into two sections (cell wall and intracellular location). Therefore, it is interesting and important to clarify which fraction carried more weight on the effect on biomass growth of rice seedlings.

For the Cr(VI)-treatments (roots), both correlations between the relative growth rate and the intracellular fraction ($R^2 = 0.92$, significant at $\alpha = 0.05$), and between the cell wall fraction and the relative growth rate ($R^2 = 0.95$, significant at $\alpha = 0.05$) were significant. Additionally, the correlation between two fractions was significant ($R^2 = 0.99$, significant at $\alpha = 0.05$). However, a partial correlation between relative growth rate, intracellular fraction and cell wall fraction indicated that the correlation between the relative growth rate and the intracellular fraction, assuming cell wall fraction a constant, would be 0.74 (significant at $\alpha = 0.05$). On the other hand, the partial correlation between the relative growth rate and the cell wall fraction (assuming intracellular fraction a constant) is sill significant ($R^2 = 0.83$) (significant at $\alpha = 0.05$). It can be concluded that both Cr factions had significant influence on relative growth rates of rice seedlings. A different conclusion was gained from the data analysis of shoot treatments with Cr(VI), which both Cr factions did not caused significant impact on relative growth rate of rice seedlings. These data analysis indicated that reduction of biomass growth of rice seedlings is largely due to Cr accumulation in roots rather than in shoots. A different conclusion was reached in Cr(III) treatments using the same data analysis, in which inhibition of relative growth rate of rice seedlings is stemmed from Cr accumulation in the intracellular fraction in shoots. Further investigation on clarification of each fraction of chemical forms from both Cr treatments will provide insight to specific role of each fraction involved in phytotoxicity of Cr in rice seedlings and identify the difference in detoxification mechanism between two Cr variants.

In conclusion, although Cr(III) was more bioavailable for rice seedlings than (VI), the latter one had higher translocation factor than the former. Additionally, both Cr variants had completely different distribution patterns in rice tissues, consequently resulted in different inhibition mechanisms, judged by a partial correlation analysis.

Author Contributions

Chun-Jiao Lu and Yu-Xi Feng performed the experiments and collected data. Xiao-Zhang Yu conceived the study, conducted data analysis and drafted the manuscript. All authors approved the final manuscript.

Conflict of Interest and Funding

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References

Aryal, R., Nirola R., Beecham, S., and Sarkar, B., 2016. Influence of heavy metals in root chemistry of *Cyperus vaginatus* R.Br: A study through optical spectroscopy. International Biodeterioration & Biodegradation, 113, 201-207. https://doi.org/10.1016/j.ibiod.2016.04.008

Cobbett, C., 2003. Heavy metals and plants-model systems and hyperaccumulators. New Phytologist, 159(2), 289-293. https://doi.org/10.1046/j.1469-8137.2003.00832.x

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

Fu, X., Dou, C., Chen, Y., Chen, X., Shi, J., Yu, M., and Xu, J., 2011. Subcellular distribution and chemical forms of cadmium in *CPhytolacca americana* L.. Journal of Hazardous

Materials, 186(1), 103-107.
https://doi.org/10.1016/j.jhazmat.2010.10.122

Lai, H.Y., 2015. Subcellular distribution and chemical forms of cadmium in *CImpatiens walleriana* in relation to its phytoextraction potential. Chemosphere, 138, 370-376.
https://doi.org/10.1016/j.chemosphere.2015.06.047

Li, C.C., Dang, F., Cang, L., Zhou, C.F., and Zhou, D.M., 2014. Integration of metal chemical forms and subcellular partitioning to understand metal toxicity in two lettuce (*CLactuca sativa* L.) cultivars. Plant Soil, 384(1-2), 201-212.
https://doi.org/10.1007/s11104-014-2194-6

Ren, J.H., Ma, L.Q., Sun, H.J., Cai, F., and Luo, J., 2014. Antimony uptake, translocation and speciation in rice plants exposed to antimonite and antimonate. Science of the Total Environment, 475(15), 83-89.
https://doi.org/10.1016/j.scitotenv.2013.12.103

Sharma, S.S., and Dietz, K.J., 2008.The relationship between metal toxicity and cellular redox imbalance. Trends in Plant Science, 14(1), 43-50.
https://doi.org/10.1016/j.tplants.2008.10.007

Sousa, A.I., Cacador, I., Lillebo, A.I., and Pardal, M.A., 2008. Heavy metal accumulation in *CHaliminoe portulacoides*: Intra- and extra-cellular metal binding sites. Chemosphere, 70(5), 850-857.
https://doi.org/10.1016/j.chemosphere.2007.07.012

Wang, X., Liu, Y.G., Zeng, G.M., Chai, L.Y., Song, X.C., Min, Z.Y., and Xiao, X., 2008. Sub-cellular distribution and chemical forms of cadmium in *Bechmeria nivea* (L.) Gaud. Environmental & Experimental Botany, 62(3), 389-395.
https://doi.org/10.1016/j.envexpbot.2007.10.014

Weng, B., Xie, X., Weiss, D.J., Liu, J., Lu, H., and Yan, C., 2012. *CKadelia obovata* (S., L.) Yong tolerance mechanisms to Cadmium: subcellular distribution, chemical forms and thiol pools. Marine Pollution Bulletin, 64(11), 2453-2460.
https://doi.org/10.1016/j.marpolbul.2012.07.047

Wu, F.B., Dong, .J, Qian, Q.Q., and Zhang, G.P., 2005. Subcellular distribution and chemical form of Cd and Cd-Zn interaction in different barley genotypes. Chemosphere, 60(10),1437-1446.
https://doi.org/10.1016/j.chemosphere.2005.01.071

Xu, P.X., and Wang, Z.L., 2013. Physiological mechanism of hypertolerance of cadmium in Kentucky bluegrass and tall fescue: Chemical forms and tissue distribution. Environmental & Experimental Botany, 96, 35-42.
https://doi.org/10.1016/j.envexpbot.2013.09.001

Yu, X.Z., Feng, Y.X., and Liang, Y.P., 2018. Kinetics of phytoaccumulation of hexavalent and trivalent chromium in rice seedlings. International Biodeterioration & Biodegradation, 128(3), 72-77.
http://dx.doi.org/10.1016/j.ibiod.2016.09.003

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.
http://dx.doi.org/10.18063/AEB.2016.01.005

Yu, X.Z., Lin, Y.J., Fan, W.J., and Lu, M.R., 2017. The role of exogenous proline in amelioration of lipid peroxidation in rice seedlings exposed to Cr(VI). International Biodeterioration & Biodegradation, 123, 106-112.
https://doi.org/10.1016/j.ibiod.2017.06.010

Yu, X.Z., and Zhang, F.F., 2017. Distribution of phenolic compounds in rice seedlings under Cr exposure. Applied Environmental Biotechnology, 2(1), 28-35.
http://dx.doi.org/10.26789/AEB.2017.01.004

Zhang, W., Lin, K., Zhou, J., Zhang, W., Liu, L., and Zhang, Q., 2014. Cadmium accumulation, sub-cellular distribution and chemical forms in rice seedling in the presence of sulfur. Environmental Toxicology & Pharmacology, 37(1), 348-353.
https://doi.org/10.1016/j.etap.2013.12.006