RESEARCH ARTICLE

Effects of trivalent chromium on biomass growth, water use efficiency and distribution of nutrient elements in rice seedlings

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Abstract: This paper presents an investigation of the effects of trivalent chromium on biomass growth (RGR), water use efficiency (WUE) and distribution of nutrient elements in young rice seedlings (*Oryza sativa* L. cv. XZX 45) exposed to chromium nitrate (Cr(III)) hydroponically. Results indicated that phytotoxicity of Cr(III) to rice seedlings was apparent, showing an linear decrease in both RGR and WUE with increasing Cr(III) concentrations. Using the Levenberg-Marquardt Algorithm, the effective concentrations (EC) obtained from the RGR were always smaller than these from WUE, indicating that the former was more sensitive to change of Cr(III) application than the latter. Although a dose-dependent total accumulation rate of Cr in plant materials was observed, the translocation of Cr into shoots was a restricted process during phyto-transport of Cr within plant materials. Results also showed that the effect of Cr(III) application on uptake and distribution of nutrient elements in rice seedlings was variable. In conclusion, the toxic response of young rice seedlings to Cr(III) was obvious and inhibitory effects were highly dependent on the total accumulation rate of Cr in plant materials.

Keywords: accumulations, effective concentration, nutrient elements, phytotoxicity, rice seedlings

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1. Introduction

During the past three decades, the rapid economic development and lack of environmental awareness has resulted in the contamination of a significant number of agricultural sites with heavy metals in mainland China[1]. Chromium (Cr) is one of most commonly found heavy metals in the environment. Indeed, it is estimated that cumulative Cr production was approximately 105.4 million tons globally in 2000[2]. Naturally, Cr(VI) and Cr(III) are the most common and stable species in the family of Cr. Both compounds are toxic, but remarkable difference in chemical properties, occurrence, behavior, and biological effects has been reported[3]. Therefore, the recommended guideline is 1 g Cr(VI)/L and 8 g Cr(III)/L for freshwater life, and 1 μg Cr(VI)/L and 50 μg Cr(III)/L for marine life[4].

The toxicity of Cr to animals and humans is well documented. It has been established that Cr(VI) is carcinogenic and mutagenic to animals and humans[5], while Cr(III) at low concentrations is considered to be a trace element essential for the proper functioning of living organisms[6]. It is well known that excessive Cr can interfere with several metabolic and biochemical processes in plants, resulting in inhibition of seed germination and plant growth, impairment of nutrient balance and water relations, degradation of photosynthetic pigments, and reduction of mitochondrial electron transport and activities of antioxidant enzymes[3,5,7,8]. Although there are abundant literatures describing phytotoxicity of Cr(VI), relatively little is known about phyto-responses to Cr(III). Additionally, numerous reports have been focused on the selection of hyperaccumulator species of plants, which mainly belong to grasses. Ample evidence showed that cultivated species may serve as a heavy metal receptor for accumulation. Rice is a global staple food, second only to wheat in its importance as a food cereal in the human diet, especially in Asia[9]. In this study, we measured several variables including biomass growth, water use efficiency and distribution of nutrient elements in order to provide more detailed information on phytotoxicity and transport of a trivalent cation in young rice seedlings exposed to chromium nitrate ranging from 2.0 mg/L (environmentally relevant) to 40.0 mg/L (high concentration).

2. Materials and Methods

2.1 Test Chemicals and Experiment Design

Fifteen-day-old rice seedlings (*Oryza sativa* L. cv. XZX 45) with similar height and weight were transplanted to a pre-treatment solution containing 1 mM $CaCl_2$ + 2 mM MES-Tris buffer (pH 6.0) for 4 hrs to clear the ions from cell wall space[10], and then ten rice seedlings were transferred into a 50 mL Erlenmeyer flask filled with 50 mL modified ISO 8692 nutrient solution[11] with addition of 10 μM Fe-EDTA, but without $NaHCO_3$. The plants were first conditioned for 24 hrs to allow adaptation to the new environmental conditions. The flasks were all wrapped with aluminum foil up to the flask mouth to prevent escape of water, and to inhibit potential growth of algae inside. All flasks were housed in a plant growth chamber with constant temperature of 25±0.5°C and a relative humidity of 60±2% under continuous artificial light. Then, the nutrient solution in each flask was replaced by spiked solution, except for the controls.

Chromium nitrate ($Cr(NO_3)_3{\cdot}9H_2O$, CAS No.7789-02-8, 99.5% purity) were purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, PR China. Eight different concentrations were used. Nominal concentrations of Cr in treatments spiked with $Cr(NO_3)_3{\cdot}9H_2O$:0, 2.0, 4.0, 8.0, 16.0, 24.0, 32.0, 40.0 mg Cr/L. Each treatment concentration was conducted in four independent biological replicates. Two testing series (48-hr and 96-hr exposure period) were conducted.

2.2 Chemical Analysis

Treated and non-treated rice seedlings were collected at the termination of experiments, rinsed with deionized water and divided into roots and shoots. Different parts of plant tissues were dried at 90°C for 48 hrs and mixed with 10 mL of 4:1HNO_3-$HClO_4$ solution for overnight. Then, samples were placed in a digestion block and heated for 2 hrs at 200°C until the digested liquid was clear. The cooled residue was dissolved in 0.2 mL of 1% HNO_3 and deionized water was added up to 50 mL of total volume[12]. The content of total Cr and other nutrient elements (K, Na, Ca, Mg, Cu, Fe, Mn and Zn) in plant materials was all analyzed by inductively-coupled plasma atomic emission spectrometry (ICP-AES).

2.3 Determination of Relative Growth Rate and Water Use Efficiency

Relative growth rate (RGR) and water use efficiency (WUE) of rice seedlings were quantified via measuring the weight prior to application and at termination of exposure, and water transpired by plants as previously described[13].

2.4 Inhibition Rate

Percent inhibition rate (IR, %) on each parameter was calculated using the equation[14]

$$IR_{(C,t)} = \left(1 - \frac{\frac{1}{n}\sum_{i=1}^{n}\mu_{(C,t)}}{\frac{1}{m}\sum_{j=1}^{m}\mu_{(O,t)}} \right) \times 100$$

Where *C* is concentration (mg Cr/L), *t* is time period (d), μ is different measured parameter, *i* is replicate 1, 2,…, *n* and *j* is control 1, 2,…, *m*.

2.5 Effective Concentration

The EC values at the respective time intervals were estimated by Levenberg-Marquardt Algorithm with 95% confidence intervals using Logistic Model of Origin v. 9.0, which is a commonly used program designed for logistic dose response in Chemistry.

$$f(x) = A_2 + \frac{A_1 - A_2}{1 + \left(\frac{x}{x_0}\right)^P}$$

Where A_1 is the initial value, A_2 is the final value, X_0 is the central value for EC of the dose-response curve, and P is the slope of dose-response curve; $f(x)$ is the function of chemical concentration x, here it refers to percent inhibition rate for each selected parameter.

2.6 Total Accumulation Rate

The total Cr accumulation rate (TAR, μg Cr/g DW.d) was calculated from final mass accumulated in different parts of plant materials using the formula[15] with slight modification

$$TAR = \frac{C_{(shoot)} \cdot DW_{(shoot)} + C_{(root)} \cdot DW_{(shoot)}}{(DW_{(shoot)} + DW_{(root)}) \cdot \Delta t}$$

Where $C_{(shoot)}$ and $C_{(root)}$ are the total Cr concentration in different plant materials and $DW_{(shoot)}$ and $DW_{(root)}$ are the dry weight production of plant materials. Δt is the time period of exposure (d).

2.7 Statistical Method

The experiments in this study were repeated four times and the data shown are the means ±SEs. One single factor ANOVA test and Tukey's multiple comparison tests were used to determine the statistical significance at the 0.05 level between Cr treatment and control.

3. Results

3.1 Effects of Cr(III) on Biomass Growth and Water Use Efficiency

Phytotoxicity of Cr(III) to young rice seedlings was obvious (Table 1), after measuring the relative growth rate (RGR) and water use efficiency (WUE). Indeed, a linear decrease in RGR was observed with increasing Cr concentrations (R^2=0.935, n=8) after 2-d incubation. Compared with non-treated plants, a significant reduction in RGR was detected with rice seedlings exposed to Cr at 4.0 mg Cr/L onwards (p<0.05), but all seedlings showed positive growth after 2-d exposure. All Cr treatments caused more remarkable effects on RGR was observed at the 4-d treatments (p<0.05) with respect to control. The change of RGR also showed a negative linear correlation (R^2=0.932, n=8).

In comparison to control, WUE in Cr-treated rice seedlings also displayed a dose-dependent decrease at the 2-d treatment (R^2=0.978, n=8) and the 4-d treatment (R^2=0.976, n=8). Decrease in WUE was all significant at higher than or equal to 8.0 mg Cr/L (p<0.05).

3.2 Determination of Effective Concentrations of Cr(III) to Rice Seedlings

The effective concentration (EC) is defined by the concentration of a chemical, which produces percentage inhibition of the maximum possible response for that chemical[16]. The inhibitory rate (%), as measured by different variables at different incubation periods are shown in Table 2. It is quite clear that higher levels of Cr(III) resulted in more severe inhibition rates on both parameters. For instance, the increased inhibitory rates ranged between 13.26% and 70.88% in response to Cr treatments (2.0 to 40.0 mg Cr/L) after 2-d exposure, with respect to control. Additionally, the inhibitory effect was dependent on duration of incubation periods. Indeed, higher inhibition rates of both RGR and WUE were observed at the treatments of 4-d exposure. It is also noted that the inhibitory rates between the two selected variables were found to be different, in which Cr(III) always caused less inhibitory

Table 1. Effects of Cr(III) concentrations (mg Cr/L) on relative growth rate (RGR, %) and water use efficiency (WUE, mg biomass/mL water transpired) of rice seedlings. Values are mean of 4 independent biological replicates. Numerical values in brackets represent standard deviation. Asterisk symbol refers to significance difference between Cr treatment and control (p < 0.05)

Conc. (mg Cr/L)		0	2.0	4.0	8.0	16.0	24.0	32.0	40.0
RGR	48-hr	23.82 (1.91)	20.66 (0.63)	19.24* (0.83)	16.25* (1.74)	13.46* (1.10)	10.02* (1.57)	8.20* (1.04)	6.94* (1.70)
	96-hr	46.84 (1.40)	37.94* (1.39)	34.89* (2.89)	28.94* (0.94)	21.86* (1.77)	16.73* (1.10)	10.28* (0.21)	7.38* (1.06)
WUE	48-hr	44.45 (3.21)	40.40 (4.28)	38.52 (3.45)	33.91* (4.04)	29.89* (3.48)	24.44* (3.89)	19.63* (4.38)	15.66* (3.12)
	96-hr	30.55 (4.53)	27.47 (3.67)	25.95 (2.74)	23.06* (1.44)	19.02* (2.36)	16.19* (0.69)	12.67* (2.33)	9.32* (2.14)

Table 2. Inhibition rate (%) of relative growth rate (RGR, %) and water use efficiency (WUE, mg biomass/mL water transpired) of rice seedling exposed to Cr(III). Values are mean of 4 independent biological replicates

Conc. (mg Cr/L)		0	2.0	4.0	8.0	16.0	24.0	32.0	40.0
RGR	48-hr	0	13.26	19.23	31.79	43.51	57.95	65.60	70.88
	96-hr	0	19.00	25.51	38.21	53.32	64.27	78.05	84.25
WUE	48-hr	0	9.12	13.34	23.71	32.75	45.01	55.85	64.77
	96-hr	0	10.08	15.06	24.51	37.74	47.02	58.53	69.47

effects on WUE than RGR in all treatments.

In this study, the EC_{10}, EC_{20} and EC_{50} correspond to the dose, at which the selected parameters are inhibited at 10%, 20% and 50% respectively[13]. Using non-linear regression, the Levenberg-Marquardt Algorithm was performed to calculate EC values. The simulation curves of concentration-response model shown in Figure 1A and 1B indicated that all trends yielded were significant, judged by the critical R for

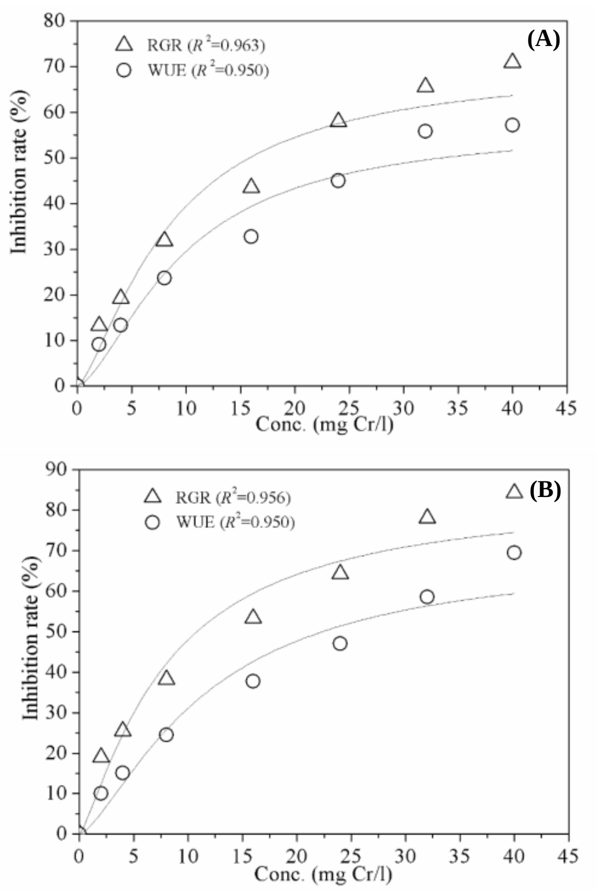

Figure 1. Simulation curves of inhibition rates of various parameters of rice seedlings exposed to different Cr(III) concentrations at different exposure periods; **(A)**: 2-d, **(B)**: 4-d; RGR: relative growth rate, WUE: water use efficiency; Values are mean of 4 independent biological replicates).

Table 3. Estimation of the ECs of Cr(III) to rice seedlings using different response endpoints

Chemicals		RGR			WUE		
		EC_{10}	EC_{20}	EC_{50}	EC_{10}	EC_{20}	EC_{50}
Cr (mg Cr/L)	48-hr	2.35	4.38	15.81	3.69	6.78	26.80
	96-hr	1.64	3.18	10.79	3.33	6.15	22.35

given n (α=0.05). Therefore, the EC values for different parameters at the respective time interval can be estimated using the fitting equations (Table 3). It is of interest to note that all EC values based on RGR were smaller than those on WUE, suggesting that RGR of rice seedlings is more sensitive to Cr(III) exposure than WUE.

3.3 Accumulation of Cr in Different Parts of Rice Seedlings

After exposure to Cr(III), total Cr content in plant materials of rice seedlings were measured (Table 4). Cr content in roots and shoots of non-treated rice seedlings was all below the detection limit, while significant amounts of Cr was found in both plant materials of Cr(III)-treated plants, indicating transport of Cr from hydroponic solution into plants and within plant materials. The total Cr content in both roots and shoots responded biphasically to Cr(III) treatment by showing linear increase at low (2.0–24.0 mg Cr/L) (root: y=60.12x+449.05, R^2=0.865; shoot: y=8.95x+26.65, R^2=0.979) and almost constants at high (24.0–40.0 mg Cr/L) concentrations for the 2-d treatment (root: mean 1816.19, SD 42.67; shoot: mean 232.65, SD 2.88). It is interesting to note that a slightly different result was obtained in the 4-d treatment. A positive linear correlation (R^2=0.963) with dose-dependent Cr content in roots was observed at 2.0–24.0 mg Cr/L of Cr(III) treatments, while a linear decrease in Cr content in roots was evident at 24.0 mg Cr/L onwards. A similar pattern in Cr content was also observed in shoots after 4 d-exposure.

3.4 Effects of Cr(III) on Distribution of Nutrient Elements

The distribution of nutrient elements in rice seedlings exposed to Cr(III) was variable. In the 2-d treatments, the macronutrient elements (K, Na, Ca and Mg) in both roots (Figure 2A) and shoots (Figure 2B) of treated rice seedlings was significantly lower than non-treated plants (p<0.05) at higher than or equal to 8.0 mg Cr/L in comparison to control, while slight

Table 4. Measured total Cr accumulated and BCF values in different plant materials of rice seedlings.

Conc. (mg Cr/L)	48-hr					96-hr				
	Cr in roots (μg/g DW)	Cr in shoots (μg/g DW)	Total accumulation rate (μg/g DW.d)	BCF in roots	BCF in shoots	Cr in roots (μg/g DW)	Cr in shoots (μg/g DW)	Total accumulation rate (μg/g DW.d)	BCF in roots	BCF in shoots
2.0	338.93 (63.39)	45.76 (11.09)	106.70 (18.66)	169.47 (31.70)	22.89 (5.55)	534.22 (65.88)	56.35 (7.31)	75.98 (9.17)	267.11 (32.94)	28.18 (3.66)
4.0	651.57 (139.18)	61.18 (13.31)	197.74 (40.74)	162.89 (34.79)	14.76 (2.92)	1072.62 (131.70)	92.49 (15.75)	129.18 (24.84)	256.46 (16.22)	23.13 (3.93)
8.0	1271.64 (153.84)	88.52 (18.96)	377.89 (38.76)	158.96 (19.23)	9.76 (3.24)	1781.38 (219.90)	150.31 (24.32)	259.13 (29.01)	228.02 (24.85)	18.79 (3.04)
16.0	1456.39 (225.40)	189.16 (53.32)	439.60 (23.85)	91.03 (14.09)	9.67 (1.87)	2464.98 (104.94)	246.98 (32.99)	362.76 (25.96)	167.77 (27.93)	18.05 (1.68)
24.0	1773.53 (153.75)	231.94 (67.16)	549.67 (57.01)	81.10 (15.32)	8.97 (2.22)	4016.15 (328.52)	319.30 (48.51)	462.82 (31.49)	139.05 (9.25)	14.94 (0.50)
32.0	1858.87 (381.62)	235.81 (90.14)	553.49 (17.46)	51.19 (16.44)	7.37 (2.82)	2635.22 (275.73)	269.16 (41.66)	358.31 (20.68)	74.15 (17.84)	8.41 (0.95)
40.0	1816.17 (143.62)	230.19 (42.61)	570.08 (41.37)	45.40 (3.59)	5.75 (1.07)	2498.63 (346.77)	263.83 (54.45)	361.58 (52.31)	62.47 (6.17)	7.20 (1.02)

Values are mean of 4 independent biological replicates. Numerical values in brackets represent standard deviation.

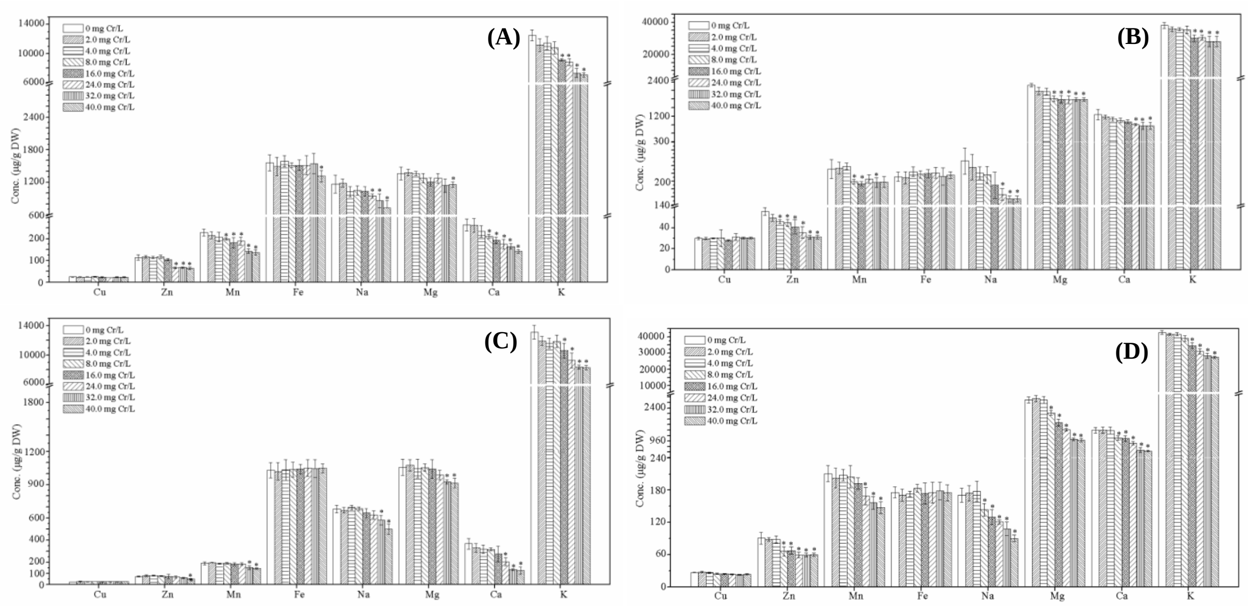

Figure 2. Content of different nutrient elements in plant materials of rice seedlings exposed to Cr(III). **(A)** Root and **(B)** shoot: 2-d; **(C)** Root and **(D)** shoot: 4-d; the values are mean of 4 individual replicates ± SDs (in brackets). Asterisk symbol refers to significant difference between the treatment and control (p<0.05).

difference between treatments and control (p>0.05) was observed below 8.0 mg Cr/L. It was remarkable that, in both control and Cr-treated rice seedlings, shoots accumulated more macronutrient elements, except for Na, than roots (p<0.05). However, a different pattern was detected in the distribution of micronutrient elements. The application of Cr(III) showed a negligible effect on Cu accumulation in both roots and shoots (p>0.05). Higher concentrations of Cr(III) caused severe effects on distribution of other micronutrient elements (Fe, Mn and Zn) (p<0.05).

Similar accumulation patterns of macronutrient elements were observed in both roots (Figure 2C) and shoots (Figure 2D) of rice seedlings in the 4-d treatments. No detectable effects was found on the content of K, Na, Ca and Mg at lower concentrations of Cr(III) (p>0.05), while higher concentrations of Cr(III) caused significant decreases in these elements in both roots and shoots in comparison to control (p<0.05).

4. Discussion and Conclusion

Inhibition of seed germination and root development, leaf-chlorosis, and stunted biomass production are the most common phytotoxic symptoms due to biotic and/or abiotic stresses[13,17]. In this work, neither negative growth in biomass nor visible toxic symptoms of chlorosis was observed in any of the Cr(III) treatments. However, a remarkable decreased trend in RGR and WUE of rice seedlings was detected with increasing Cr(III) concentrations. We also noted that both parameters showed differently responsive to Cr(III) exposure, in which RGR of rice seedlings was more sensitive to Cr change than WUE, judged by the lower ECs values. A similar conclusion was also reached in our previous work, where a lower ECs values for rice seedlings exposed to Cd was obtained using RGR as sensitive variables in comparison to WUE[13].

The bioconcentration factor (BCF) is defined as the ratio of metal concentration in the biomass to the initial concentration of metal ions in the feed solution[18]. The estimation of BCF values from roots and shoots of rice seedlings exposed to Cr(III) are shown in Table 4, in which significantly higher BCF values in roots than in shoots were obtained (p<0.05) in both testing series, indicating that the translocation of Cr within plant materials was most likely to occur, but roots rather than shoots were major sites for Cr accumulation. It was evident that rice seedlings were able to take up more Cr from the hydroponic solution after exposing rice to Cr(III) for 4 d. Indeed, the BCF values in both roots and shoots at the 4-d treatment were always higher than these at the 2-d treatment (p<0.05). Here, interests have been generated to compare the translocation potential between the two testing series using the ratio of BCF_{root} to BCF_{shoot} at the respective treatment concentrations. This outcome of the comparison was quite surprising: a very similar ratio was obtained in both treatments, where the mean values were determined to be 9.72 (SD 3.21) and 9.83 (SD 1.29) for 2-d and 4-d treatments, respectively, suggesting that the translocation capacity of Cr from roots to shoots was independent on the exposure period.

It is evident that excess of heavy metals usually affects mineral nutrient homeostasis, which results from the effects on availability, absorption and transport of nutrients within plants[11,19]. Indeed, uptake and distribution of nutrient elements was variable in rice seedlings exposed to Cr(III), most likely due to their different roles in plant growth, development and yield[11]. For instance, K is an important enzyme activator involved in synthesis of protein and sugar, and also functions in osmotic modulation in plants[20]. In this current work, a significant difference in K content in both roots and shoots was observed in rice seedlings to Cr(III) at concentrations of 8.0 mg Cr/L onwards in respect to control. This is constant to the results obtained from estimation of RGR and WUE. Mg is a key component of chlorophyll, and Cu, Fe and Mn play a vital role in synthesis or stability of chlorophyll[21]. However, uptake and distribution of these nutrient elements was completely different in this work. Negligible effects on Mg, Fe and Cu were observed, while the application of Cr(III) showed significantly impact on distribution of Mn, suggesting that Cr(III) may carry more weight on the effect on uptake and distribution of Mn rather than Cu, Fe and Mg. Additionally, Zn is the only metal represented in several electron transport enzymes[22]. In general, higher doses of Cr(III) had severe effects on uptake and distribution of Zn in both roots and shoots.

In summary, phytotoxicity of Cr(III) to young rice seedlings was evident, judged by negative responses of both RGR and WUE to Cr exposure. Results from EC estimation indicated that the former was more susceptible to the changes of Cr(III) than the latter. Although rice seedlings were able to take up Cr(III) efficiently, translocation of Cr within plant materials was a restricted process. Additionally, the effect of Cr(III) application on uptake and distribution of nutrient elements was variable.

Author Contributions

Xing-Hui Feng 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

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