

Model for Evaluation of the Phytoavailability of Chromium (Cr) to Rice (*Oryza sativa* L.) in Representative Chinese Soils

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ABSTRACT: Anthropogenic chromium (Cr) pollution in soils poses a great threat to human health through the food chain. It is imperative to understand Cr phytoavailability to rice (*Oryza sativa* L.), which is a major staple food crop for the largest population of people on Earth. This study was aimed to establish a model for evaluation of the phytoavailability of Cr to rice in six representative Chinese soils based on soil properties. Simple correlation analysis indicated that Cr concentration in polished rice was significantly correlated with total Cr, Mehlich-3 extractable Cr, and Cr(VI) in soil. Stepwise multiple regression analysis also demonstrated that the Cr phytoavailability was strongly correlated with soil total Cr, Mehlich-3 extractable Cr, Cr(VI) concentration, soil organic matter, Fe(II), and particle size distribution. Critical Cr concentrations in the six soils were evaluated for rice based on the maximum safe level for daily intake of Cr. Mehlich-3 extractable Cr are the most suitable Cr thresholds for Periudic Argosols, Udic Ferrisols, Mollisols, and Ustic Cambosols with values of 1.54, 0.56, 0.42, and 2.18 mg kg⁻¹, respectively, while Cr(VI) are adequate thresholds for Calcaric Regosols and Stagnic Anthrosols with values of 0.68 and 0.84 mg kg⁻¹, respectively.

KEYWORDS: chromium thresholds, food safety, phytoavailability, soil properties, *Oryza sativa* L

■ INTRODUCTION

As the 21st most abundant element in Earth's crust, chromium (Cr) has been extensively used in industrial activities such as ore refining, electroplating industry, tanning, paper making, steel production, and automobile manufacturing.¹ As a consequence, there is a continual influx of Cr contaminants into the environment. The lack of appropriate disposal facilities has led to severe Cr pollution in waters and soils throughout the world.² Accumulation of Cr in soils increases the potential uptake of the metal by plants, thus posing a great threat to human health through the food chain.³ As rice (*Oryza sativa* L.) is the major staple food crop for nearly 40% of the world population and more than 60% of the population in China,⁴ it is one of the most important pathways through which heavy metals enter the food chain and affect human health. It is therefore imperative to control Cr concentrations in rice, especially in polished rice to ensure food safety. To limit the accumulation of Cr in rice, a good understanding of its accumulation properties is crucial. There is a raised concern over Cr pollution in food and potential risks to human health.^{5,6}

In the natural environment, Cr exists in two common oxidation states: Cr(III) and Cr(VI). Chromium(III), an essential trace element for mammals,⁷ has been shown to be nontoxic to benthic macroinvertebrates at concentrations approaching 2% of dry weight⁸ and is generally considered immobile and nonbioavailable due to the low solubility of Cr(III) (hydr)oxides at neutral pH.⁹ Conversely, Cr(VI) exists as highly soluble oxyanionic species, i.e., CrO₄²⁻ (chromate), HCrO₄⁻ (bichromate), and Cr₂O₇²⁻ (dichromate), is a known human carcinogen,¹⁰ and elicits acute and chronic toxicological responses in benthic organisms.⁸

The mobility, toxicity, and plant uptake of Cr depend strongly on its oxidation states.

Reduction and oxidation of Cr have been demonstrated to be associated with soil properties, including the content of electron donors [organic matter (OM),¹¹ Fe(II),¹² Mn(II)],¹³ electron acceptors [Mn(III, IV) oxides],¹⁴ texture, and pH of the soil.¹⁴ Therefore, in a soil–plant system, risk assessments include physicochemical characteristics of soils, e.g., OM, pH, Fe(II), Mn(II), easily reducible Mn [Mn(ER)], cation exchange capacity (CEC), and particle size distribution (PSD).

The U.S. National Academy of Sciences has proposed an estimated safe and adequate daily dietary intake (ESADDI) for Cr.¹⁵ The need to protect consumers from Cr toxicity and ensure environmental safety is the scientific motive for establishing acceptable concentrations of Cr in agricultural soils. The objectives of this study were thus as follows: (1) to evaluate the combined effect of soil properties on the phytoavailability of Cr to rice (*Oryza sativa* L.); an empirical model was developed to correlate the Cr phytoavailability with common soil properties and with Cr concentrations in soil extractable fractions) and (2) to establish Cr thresholds for potential dietary toxicity in representative agricultural soils.

■ MATERIALS AND METHODS

Soil Samples Collection and Preparation. Six representative agricultural soils were used in this study, classified as Udic Ferrisols,

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Table 1. Physicochemical Properties of the Soils

soil	Periudic Argosols	Udic Ferrisols	Calcaric Regosols	Stagnic Anthrosols	Mollisols	Ustic Cambosols
chromium (mg kg ⁻¹)						
total Cr (mg kg ⁻¹)	30.2 ± 2.5 c	68.5 ± 2.5 a	58.0 ± 2.2 b	56.8 ± 2.9 b	65.5 ± 2.0 a	35.8 ± 1.8 c
chemical characteristics ^a						
pH	5.37 ± 0.08 e	5.03 ± 0.05 f	8.25 ± 0.07 a	6.49 ± 0.04 d	7.23 ± 0.03 c	7.80 ± 0.02 b
OM (g kg ⁻¹)	11.6 ± 0.3 d	19.1 ± 0.6 c	21.8 ± 0.2 b	21.4 ± 0.1 bc	32.2 ± 0.3 a	7.54 ± 0.20 e
CEC (cmol kg ⁻¹)	12.6 ± 1.5 d	17.3 ± 2.0 cd	25.5 ± 2.1 b	20.2 ± 1.4 c	34.0 ± 2.5 a	15.8 ± 1.6 d
Fe(II) (mg kg ⁻¹)	34.3 ± 0.8 b	34.4 ± 1.5 b	30.2 ± 0.8 c	42.4 ± 0.9 a	30.5 ± 0.6 c	27.6 ± 0.5 d
Mn(ER) (mg kg ⁻¹)	134 ± 7 c	2.64 ± 0.23 e	264 ± 10 a	231 ± 9 b	109 ± 5 d	140 ± 6 c
soil texture						
sand (%)	24.8 ± 0.7 b	10.6 ± 0.2 d	31.6 ± 1.0 a	11.4 ± 0.3 d	20.6 ± 1.5 c	21.6 ± 1.3 c
silt (%)	58.2 ± 1.0 c	39.8 ± 1.3 d	44.0 ± 1.3 c	73.0 ± 2.4 a	60.2 ± 2.2 bc	65.4 ± 2.6 b
clay (%)	17.0 ± 0.3 c	49.6 ± 1.2 a	24.4 ± 0.8 b	15.6 ± 1.2 c	19.2 ± 1.2 c	13.0 ± 1.1 c

^aMean values followed by different letters (a–f) within the same row are significantly different at $P < 0.05$. CEC, cation exchange capacity; Mn(ER), easily reducible Mn; OM, organic matter.

Periudic Argosols, Calcaric Regosols, Stagnic Anthrosols, Mollisols, and Ustic Cambosols according to Chinese Soil Taxonomic Classification.¹⁶ They were, respectively, collected (at the 0–20 cm depth) from Guilin City (104°40′–119°45′E, 24°18′–25°41′N), Huzhou City (119°68′–120°43′E, 30°53′–31°02′N), Ya'an City (102°37′–103°12′E, 29°23′–30°37′N), Jiaxing City (120°7′–121°02′E, 30°5′–30°77′N), Ha'erbing City (126°32′–129°55′E, 44°92′–46°32′N), and Qufu City (116°51′–117°13′E, 35°29′–35°49′N), China. After removal of visible pieces of plant materials, earthworms, grit, etc., the fresh soil samples were analyzed for Fe(II) contents¹⁷ and Mn(ER) contents.¹⁸ Subsamples of soil were air dried, ground, passed through a 2 mm sieve, and analyzed for pH,¹⁹ CEC,²⁰ OM contents,²¹ and PSD.²² Relevant physicochemical properties of the soils are shown in Table 1.

Soil samples (Periudic Argosols and Ustic Cambosols) with background values (BV) of Cr concentration less than 50 mg kg⁻¹ were spiked with Cr as K₂Cr₂O₇ (with purity > 98% from Aldrich Chemical Co.) to establish contaminant levels of BV, 50, 100, 200, 300, and 400 mg Cr kg⁻¹ soil. On the other hand, soil samples (Udic Ferrisols, Calcaric Regosols, Stagnic Anthrosols, and Mollisols) with background values (BV) of Cr concentration above 50 mg kg⁻¹ were spiked with K₂Cr₂O₇ to establish contaminant levels of BV, 100, 200, 300, and 400 mg Cr kg⁻¹ soil. All spiked soil samples were aged for 1 year at a moisture content of 70% of water holding capacity prior to pot experiments. At the end of preincubation, the concentrations of total Cr, Mehlich-3 extractable Cr, and Cr(VI) were determined.

Pot Experiment. The rice (*Oryza sativa* L.) variety used was Zhongzheyou 1, which is a single-season indica variety with an average plant height of 120 cm. This long duration variety takes about 140 days to mature. Seed of the variety was obtained from the Zhejiang Seed Co. Seeds were surface sterilized by washing with 70% ethanol for 1 min and soaking in 0.01 g mL⁻¹ sodium hypochlorite for 5 min, rinsed thoroughly in deionized water, and then imbibed in deionized water for 48 h at 30 °C.²³ Then seeds were germinated in quartz sand washed with 5% (v/v) HCl. For the first 2 weeks, only deionized water was supplied. After 14 days when seedlings grew onto two-leaf stage, nutrient solution was supplied. The composition of nutrient solution was the same as that described by Yang et al.²⁴ On May 30, 2012, 4 seedlings (30 days old) were transplanted to individual pots for each treatment. All treatments were conducted in triplicate, and pots were randomly arranged in a greenhouse under a photo flux density of 400 μmol m⁻² s⁻¹, a light/dark period of 16/8 h, day/night temperatures of 30/25 °C, and day/night relative humidity of 75/85%.²⁵ Before transplanting, the standard recommended dose of NPK fertilizer was applied to all pots at rates of 187.5 kg N ha⁻¹ (70% applied as basal dose and 30% as topdressing at panicle initiation stage), 70 kg P₂O₅ ha⁻¹, and 93 kg K₂O ha⁻¹.²⁶ Water management was the same as the conventional rice-farming practice.

Plants were harvested at maturity and oven dried. Plant samples were manually threshed to separate grains; then the oven-dried

weights of grains and straws were recorded. Brown rice was prepared by removing the husk using a laboratory dehusker (JLGJ4.5, Taizhou Cereal and Oil Instrument Co. Ltd., Zhejiang, China); polished rice was prepared by polishing the bran by a laboratory polishing machine (JNMJ3, Taizhou Cereal and Oil Instrument Co. Ltd., Zhejiang, China). The husk, brown rice, and polished rice samples were ground using a ball mill (Retsch, MM-301, Germany) and passed through a 60-mesh sieve and then kept at –20 °C prior to Cr analysis.

Chemical Analysis. *Total Cr in Soil and Plant.* For determination of total Cr in soil, portions of each 0.20 g of soil samples were digested with HNO₃–HClO₄–HF (5:1:1).²⁷ Rice samples (0.1 g) of each treatment were digested with HNO₃–H₂O₂ (4:1), and the digested solution was transferred to a 50 mL volumetric flask, made up to volume, and filtered.²³ Concentrations of Cr in the filtrate were determined using inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500a) with a detection limit of 0.3–0.4 μg Cr L⁻¹. ICP-MS was operated at the following conditions: radiofrequency power at the torch 1.2 kW, plasma gas flow 15 L min⁻¹, auxiliary gas flow 0.89 L min⁻¹, and carrier gas flow 0.95 L min⁻¹.²⁸ Validation of the presented procedure was checked by analysis of two certified reference materials (soil GSBZ 50013-88 and rice NCSZC73008) approved by General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ), with a recovery rate of 96.7% and 103.4%, respectively. Three replications were conducted for each sample.

Mehlich-3 Extractable Cr in Soils. Soil Mehlich-3 extractable Cr was determined following the extraction procedure described by Mehlich.²⁹ Briefly, fresh soil in portions equivalent to 2.5 g of oven-dry weight was shaken with 25 mL of Mehlich-3 solution (0.2 mol L⁻¹ CH₃COOH, 0.25 mol L⁻¹ NH₄NO₃, 0.015 mol L⁻¹ NH₄F, 0.013 mol L⁻¹ HNO₃, 0.001 mol L⁻¹ EDTA) for 5 min (200 rpm) at 25 °C, and then the suspension was centrifuged at 4000g for 10 min and filtered through 0.45 μm filter membrane. Cr concentration in filtrate was analyzed by ICP-MS. Quality assurance and quality control (QA/QC) for extractable Cr in soil samples were conducted by determining Cr contents in the standard reference material GBW07443 (GSF-3) approved by AQSIQ with a recovery rate of 95.6%. Three replications were conducted for each sample.

Cr(VI) in Soils. Extraction and analysis of soil samples for Cr(VI) were conducted according to the modified EPA Method 3060A.³⁰ Fresh soil samples (2.5 g) were digested with 50 mL of 0.28 M Na₂CO₃/0.5 M NaOH in 250 mL digestion vessels. Solutions were heated at 95 °C for 60 min with continuous stirring. After cooling, the digested suspension was filtered and the filter cake was washed twice with 5 mL of digesting solution. Filtrates were neutralized with nitric acid to a pH of 7–8 and then diluted to 100 mL with deionized water. Concentrations of Cr in the solutions were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES, Thermo iCAP 6300). The ICP-OES was composed of an EMT duo quartz torch, glass spray chamber, and concentric glass nebulizer and operated at the following conditions: radiofrequency power at the

torch 1.15 kW, plasma gas flow 12 L min⁻¹, auxiliary gas flow 1 L min⁻¹, nebulizer gas flow 0.5 L min⁻¹, and integration time 30 s.³¹ The instrumental detection limit for Cr was 0.9 µg mL⁻¹. Experiments on Cr(VI) recovery were carried out by adding known concentrations of Cr(VI) standards (10 and 100 mg kg⁻¹) to Cr(VI) free soil. Recovery of spiked Cr(VI) was 93.5 ± 2.9% and 96.3 ± 4.7%, respectively. Three replications were conducted for each sample.

Statistical Analyses. Means of data were compared by the least significant difference (LSD) tests at the 5% significance level. One-way analysis of variance (ANOVA), linear regression, and multiple regression analysis were performed using the statistical package SPSS 18.0 for Windows (CoHort Software, Berkeley, CA).

RESULTS

Characteristics of Soils. There were significant differences in soil properties that influence the reduction of Cr(VI) among the six soils and Cr accumulation in rice (Table 1). Concentrations of Cr(VI) in all studied soils were very low, ranging from 0.26 mg kg⁻¹ for the Calcaric Regosols to 0.50 mg kg⁻¹ for the Stagnic Anthrosols, whereas total Cr concentrations (background value) in the soils ranged from 30.2 to 68.5 mg kg⁻¹.

Soil pH ranged from 5.03 for the Udic Ferrisols to 8.25 for the Calcaric Regosols, i.e., strong acid to mild alkaline, total OM content from 7.54 g kg⁻¹ for the Ustic Cambosols to 32.2 g kg⁻¹ for the Mollisols, and CEC from 12.6 cmol kg⁻¹ for the Peridic Argosols to 34.0 cmol kg⁻¹ for the Mollisols. Fe(II) and Mn(ER) contents were the lowest in the Ustic Cambosols (27.6 mg kg⁻¹) and Udic Ferrisols (2.64 mg kg⁻¹), while the highest values were found in the Stagnic Anthrosols (42.4 mg kg⁻¹) and Calcaric Regosols (264 mg kg⁻¹). The clay and silt fractions of all soils were relatively high, from 13.0% for the Ustic Cambosols to 49.6% for the Udic Ferrisols and from 39.8% for the Udic Ferrisols to 73.0% for the Stagnic Anthrosols.

Mehlich-3 Extractable Cr and Cr(VI) after Aging. Mehlich-3 extractable Cr and Cr(VI) after aging for 1 year increased with the initial soil Cr loading rates (Table 2). The relationships between soil total Cr and Mehlich-3 extractable Cr as well as Cr(VI) were both better described by a quadratic ($R^2 > 0.97$) than a linear equation ($r^2 > 0.91$), suggesting that Cr(VI) reduction in soil was slowed down at higher Cr concentrations. Mehlich-3 extractable Cr and Cr(VI) were 0.13–12.9 and 0.28–15.2 mg kg⁻¹, respectively, in all soils. The Mehlich-3 extractability of Cr varied significantly among the six soils and decreased in the order Ustic Cambosols > Peridic Argosols > Mollisols > Calcaric Regosols > Stagnic Anthrosols > Udic Ferrisols (Table 2).

Stepwise regression models for predicting Mehlich-3 extractable Cr and Cr(VI) after aging for 1 year at a Cr rate of 400 mg kg⁻¹ were conducted based on soil characteristics (Table 3). Out of the variables measured, four were extracted as being significant for both regression models. The extracted variables included pH, OM, Fe(II), and clay fractions (Table 3). Both coefficients of multiple correlation and partial regression reached at least the 0.05 significance level.

Biomass Yield of Rice. Dry weights (DWs) of rice in relation to external Cr loading rates are summarized in Table 4. As compared to the nonspiked soils, growth of rice plants appeared to be promoted at a Cr rate of 100 mg kg⁻¹ in Udic Ferrisols, Calcaric Regosols, Stagnic Anthrosols, and Mollisols, but the differences in grain and straw yield were not statistically significant. Dry weights of rice decreased gradually as the Cr rate increased, up to 32.8–78.2% of the control at 400 mg kg⁻¹ in all soils. Growth inhibition might be attributed to Cr

Table 2. Mehlich-3 Extractable Cr and Cr(VI) at the Beginning of Pot Experiment after Aging for 1 Year (mg kg⁻¹)^a

	Peridic Argosols		Udic Ferrisols		Calcaric Regosols		Stagnic Anthrosols		Mollisols		Ustic Cambosols	
	C _{rExt} ^b	Cr(VI)	C _{rExt}	Cr(VI)	C _{rExt}	Cr(VI)	C _{rExt}	Cr(VI)	C _{rExt}	Cr(VI)	C _{rExt}	Cr(VI)
Cr-CK ^c	0.28 ± 0.02 f	0.45 ± 0.03 f	0.18 ± 0.02 e	0.28 ± 0.01 e	0.13 ± 0.02 e	0.26 ± 0.02 a	0.28 ± 0.04 e	0.50 ± 0.05 e	0.33 ± 0.02 e	0.45 ± 0.01 e	0.29 ± 0.02 f	0.40 ± 0.03 f
Cr-50	1.61 ± 0.18 e	2.20 ± 0.41 e	0.81 ± 0.14 d	1.21 ± 0.29 d	1.05 ± 0.24 d	1.87 ± 0.67 b	1.06 ± 0.34 d	1.43 ± 0.47 d	1.22 ± 0.46 d	2.44 ± 0.98 d	2.32 ± 0.71 e	3.11 ± 0.45 e
Cr-100	3.52 ± 0.22 d	5.17 ± 0.69 d	2.04 ± 0.21 c	2.67 ± 0.14 c	2.72 ± 0.73 c	3.13 ± 0.66 c	2.33 ± 0.73 c	2.95 ± 0.85 c	3.26 ± 0.92 c	5.11 ± 0.13 c	5.81 ± 0.81 d	6.78 ± 1.22 d
Cr-200	5.21 ± 0.58 c	7.73 ± 0.85 c	2.82 ± 0.41 b	3.87 ± 0.24 b	4.12 ± 0.82 b	5.36 ± 0.95 b	3.19 ± 0.81 b	4.89 ± 0.62 b	5.12 ± 0.81 b	7.54 ± 1.17 b	8.23 ± 1.07 c	9.64 ± 1.31 c
Cr-300	8.04 ± 0.72 b	9.32 ± 1.73 b	4.05 ± 0.04 a	5.34 ± 0.15 a	8.38 ± 0.88 a	9.52 ± 1.09 a	7.03 ± 0.76 a	7.85 ± 0.96 a	9.24 ± 1.10 a	10.1 ± 1.2 a	10.5 ± 1.5 b	12.4 ± 1.6 b
Cr-400	11.2 ± 1.0 a	12.5 ± 1.2 a									12.9 ± 1.6 a	15.2 ± 1.3 a

^aMean values followed by different letters (a–f) within the same column are significantly different at $P < 0.05$. ^bC_{rExt} refers to Mehlich-3 extractable Cr. ^cCr-CK refers to soil samples without spiking Cr.

Table 3. Stepwise Regression Models for Predicting Mehlich-3 Extractable Cr and Cr(VI) at Initial Soil Cr Loading Rate of 400 mg kg⁻¹ after Aging for 1 Year Based on Soil Characteristics (n = 32)

stepwise regression model ^a	R ²	F value ^b		T value ^b of the partial regression coefficient	R ² of the partial regression coefficient
Y ₁ = 31.6 - 0.711pH - 0.0719OM ^c - 0.359Fe(II) - 0.207clay content	0.999	1019.4**	pH	11.9**	0.997
			OM	11.2**	0.996
			Fe(II)	28.6**	0.999
			clay content	43.0**	0.999
Y ₂ = 34.8 - 0.651pH - 0.119OM - 0.402Fe(II) - 0.204clay content	0.998	188.8*	pH	4.23*	0.973
			OM	7.21*	0.991
			Fe(II)	12.5**	0.997
			clay content	16.5**	0.998

^aY₁ = the amount of Mehlich-3 extractable Cr; Y₂ = the amount of Cr(VI). ^bSuperscripts * and ** indicate significant level at 0.05 and 0.01, respectively. ^cOM, organic matter.

toxicity.³² Significant differences in the growth of rice were found among the six soil types, and the DWs of rice at 400 mg kg⁻¹ generally decreased in the order Calcaric Regosols > Udic Ferrisols > Stagnic Anthrosols > Mollisols > Periudic Argosols > Ustic Cambosols (Table 4).

Distribution of Cr in Rice. Chromium concentrations in rice tissues varied with Cr levels and type of soils and increased with increasing Cr loading rates (Figure 1). Chromium concentration in rice was in the order of husk > brown rice > polished rice and ranged from 0.58 to 27.3, 0.53 to 21.2, and 0.46 to 18.4 mg kg⁻¹, respectively (Figure 1). Chromium accumulation in rice tissues was significantly affected by soil type, mainly due to the difference in Cr phytoavailability among the six soils. Chromium concentrations in rice conformed to an order of Ustic Cambosols > Periudic Argosols > Mollisols > Calcaric Regosols > Stagnic Anthrosols > Udic Ferrisols at the same Cr loading rate (Figure 1).

Chromium concentration in the edible parts (polished grain) of rice plants was positively correlated with soil total Cr ($r^2 > 0.882$), Mehlich-3 extractable Cr ($r^2 > 0.963$), and Cr(VI) content ($r^2 > 0.886$), which could be well described by a linear equation (Table 5). Chromium concentrations in polished rice were best correlated to Mehlich-3 extractable Cr in Periudic Argosols, Udic Ferrisols, Mollisols, and Ustic Cambosols, with r^2 values of 0.966, 0.977, 0.993, and 0.984, respectively, while Cr concentrations of polished rice in Calcaric Regosols and Stagnic Anthrosols were best correlated to soil Cr(VI) content, with r^2 values of 0.987 and 0.995, respectively.

DISCUSSION

Relationship between Soil Properties and Mehlich-3 Extractable Cr and Cr(VI). Although the Cr loading rates were the same, the contrasting concentrations of Mehlich-3 extractable Cr and Cr(VI) after aging for 1 year were observed in different soils studied. As shown in Table 1, pH, OM, CEC, Fe(II), Mn(ER), and PSD differed considerably among the six soils. Thus, the variations in the concentrations of Mehlich-3 extractable Cr and Cr(VI) after the aging period might be ascribed to the differences in the composition and properties of the tested soils. This hypothesis was confirmed by the results of stepwise regression, which indicates the relative importance of pH, OM, Fe(II), and PSD for reduction of Cr(VI) and extractability of Cr in soil (Table 3). This finding was in agreement with previous reports.^{12,14,33}

Organic matter, which has been identified to facilitate reduction of Cr(VI) to Cr(III) in soils, represents a significant

reservoir of electron donors for Cr(VI) reduction.¹⁴ Also, organic carbon fractions provide the energy source for the soil microorganisms involved in reduction of Cr [i.e., Cr(VI) to Cr(III)].³⁴

In addition to OM, Fe(II) is the most common reductant involved in reduction of Cr(VI) in soil.¹² Mineral phases that contain significant amounts of Fe(II) such as magnetite (Fe₃O₄), pyrite (FeS₂), biotite (black mica), etc., are known to reduce Cr(VI).³⁵ Particulate Fe oxyhydroxides act as electron donors and release Fe(II) in the presence of organic ligands, and Fe(II) then reduces Cr(VI) to Cr(III).¹⁴ Buerge and Hug³⁶ reported that Fe(II) promotes Cr(VI) reduction by natural organic material as a redox catalyst.

pH plays an important role in the environmental behavior of Cr by controlling the distribution of the Cr(VI) species. pH may enhance adsorption of HCrO₄⁻ or increase the rates of electron transfer for adsorbed HCrO₄⁻ relative to adsorbed CrO₄²⁻. Rates of Cr(VI) reduction increase with decreasing pH due to increased protonation level of Cr(VI) species.³³ The enhanced rates of Cr(VI) reduction by Fe(II)-bearing minerals at a low pH are related to enhanced rates of minerals dissolution and reaction of Cr(VI) with dissolved Fe(II).³⁷

Soil particles consist of different minerals with different chemical formulas, supplying sites for most chemical, physical, and biological activities.³⁸ Although few studies have reported the contribution of soil PSD to Cr(VI) reduction, Loyaux-Lawniczak et al.³⁹ noted that clay particles can be considered as the Cr-Fe-bearing phase in the finest fraction, suggesting reaction between Cr(VI) and Fe(II) took place in the clay fraction. Kwok and Loh⁴⁰ also reported that the small size particles, especially the clay particles, result in large surface area-to-mass ratios to supply the sites for microbial activities.

Chromium(VI) exists as highly soluble oxyanionic species,¹⁴ but conversely, Cr(III) is generally considered immobile due to its low solubility in water at a normal pH range (4–9).⁹ Therefore, this differentiation in Mehlich-3 extractable Cr among the six soils might be attributed to the different rates of Cr(VI) reduction. From the above results, it was concluded that soil OM, Fe(II), pH, and PSD could remarkably influence Cr(VI) reduction rates and Cr extractability in soil. Furthermore, soil properties display more complicated effects on the phytoavailability of Cr to plants. Thus, it is very important to investigate the combined effects of these factors on the phytoavailability of Cr to rice.

Multiple Regression Model for Evaluating Cr Phytoavailability. Principally, the extraction methods were based on

Table 4. Dry Weight of Rice Grown on Representative Chinese Soils with Different Loading Rates of Cr (g pot⁻¹)

	Periudic Argosols		Udic Ferrisols		Calcaric Regosols		Stagnic Anthrosols		Mollisols		Ustic Cambosols	
	grain	straw	grain	straw	grain	straw	grain	straw	grain	straw	grain	straw
Cr-CK ^a	37.5 ± 1.8 a	34.7 ± 1.6 a	42.1 ± 1.6 a	39.6 ± 1.1 a	44.5 ± 1.9 a	41.1 ± 1.5 a	45.3 ± 2.9 a	43.3 ± 1.4 a	48.7 ± 2.0 a	44.1 ± 1.5 a	35.4 ± 1.2 a	33.2 ± 1.3 a
Cr-50	32.1 ± 1.3 b	29.5 ± 1.7 b	42.6 ± 1.8 a	40.3 ± 1.6 a	44.8 ± 1.6 a	41.8 ± 1.8 a	47.8 ± 2.3 a	44.5 ± 1.6 a	49.1 ± 1.6 a	44.9 ± 2.4 a	30.9 ± 1.0 b	28.1 ± 1.5 b
Cr-100	26.3 ± 1.3 c	24.3 ± 1.4 c	39.8 ± 1.5 a	37.7 ± 1.6 a	41.5 ± 1.8 a	39.1 ± 1.3 a	39.0 ± 2.5 b	36.2 ± 1.3 b	41.2 ± 1.8 b	38.1 ± 1.9 b	24.6 ± 1.5 c	23.2 ± 1.6 c
Cr-200	22.5 ± 1.5 d	19.8 ± 1.0 d	35.0 ± 1.4 b	32.3 ± 1.6 b	37.1 ± 1.5 b	34.9 ± 1.7 b	33.1 ± 2.7 c	28.4 ± 1.3 c	32.6 ± 2.0 c	27.2 ± 1.5 c	20.4 ± 1.0 d	18.1 ± 1.3 d
Cr-300	18.8 ± 1.6 e	16.2 ± 1.1 e	32.4 ± 1.4 b	29.4 ± 1.8 b	34.8 ± 1.6 b	31.8 ± 1.5 b	24.5 ± 2.1 d	21.5 ± 1.0 d	22.6 ± 1.5 d	20.3 ± 1.2 d	16.3 ± 1.4 e	14.5 ± 1.2 e
Cr-400	15.1 ± 1.2 f	12.5 ± 1.2 f									12.2 ± 1.2 f	10.9 ± 1.7 f

^aMean values followed by different letters (a–f) within the same column are significantly different at *P* < 0.05. Cr-CK refers to soil samples without spiking Cr.

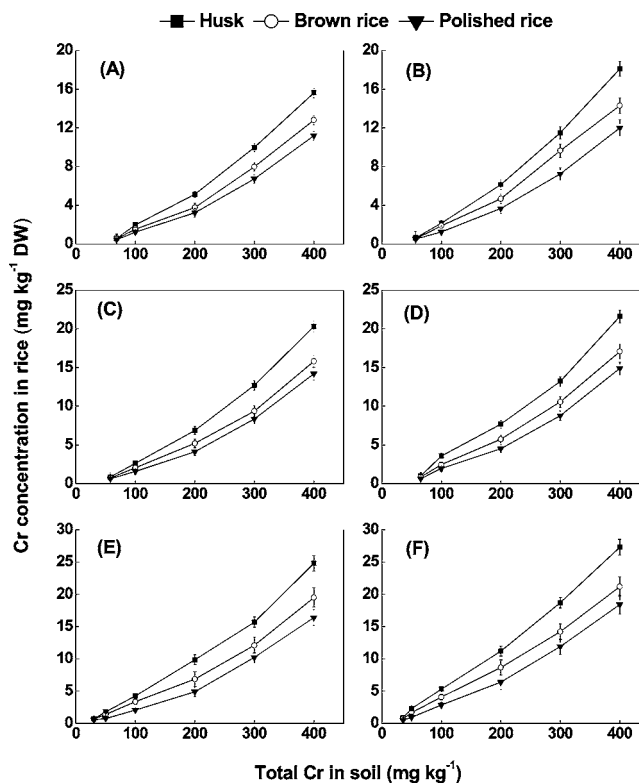


Figure 1. Chromium distribution in rice grown on Udic Ferrisols (A), Stagnic Anthrosols (B), Calcaric Regosols (C), Mollisols (D), Periudic Argosols (E), and Ustic Cambosols (F) spiked with different concentrations of Cr.

Table 5. Regression Analysis between Cr Concentrations in Polished Rice (*y*) and Soil Cr Concentrations (*x*)

soil type	soil Cr	regression equation	r ²
Periudic Argosols	total Cr	<i>y</i> = 0.042 <i>x</i> - 1.759	0.955
	Mehlich-3 extractable Cr	<i>y</i> = 1.509 <i>x</i> - 1.698	0.966
	Cr(VI)	<i>y</i> = 1.308 <i>x</i> - 2.336	0.874
Udic Ferrisols	total Cr	<i>y</i> = 0.031 <i>x</i> - 2.191	0.950
	Mehlich-3 extractable Cr	<i>y</i> = 2.785 <i>x</i> - 0.945	0.977
	Cr(VI)	<i>y</i> = 2.131 <i>x</i> - 1.128	0.950
Calcaric Regosols	total Cr	<i>y</i> = 0.038 <i>x</i> - 2.450	0.966
	Mehlich-3 extractable Cr	<i>y</i> = 1.707 <i>x</i> + 0.177	0.983
	Cr(VI)	<i>y</i> = 1.542 <i>x</i> - 0.435	0.987
Stagnic Anthrosols	total Cr	<i>y</i> = 0.033 <i>x</i> - 2.057	0.973
	Mehlich-3 extractable Cr	<i>y</i> = 1.769 <i>x</i> + 0.023	0.963
	Cr(VI)	<i>y</i> = 1.611 <i>x</i> - 0.739	0.995
Mollisols	total Cr	<i>y</i> = 0.041 <i>x</i> - 2.586	0.972
	Mehlich-3 extractable Cr	<i>y</i> = 1.626 <i>x</i> - 0.070	0.993
	Cr(VI)	<i>y</i> = 1.456 <i>x</i> - 1.304	0.944
Ustic Cambosols	total Cr	<i>y</i> = 0.048 <i>x</i> - 1.866	0.882
	Mehlich-3 extractable Cr	<i>y</i> = 1.380 <i>x</i> - 2.395	0.984
	Cr(VI)	<i>y</i> = 1.190 <i>x</i> - 2.614	0.886

the assumption that there was a good relationship between the extractable fraction of metals and uptake of the metals by plants, and the good relationship reflected that the extractable fraction of the metals in soils was available to plants.⁵ The Mehlich-3 extractant was reliable for evaluating Cr phytoavailability to rice in the tested soils, as evidenced by the high correlation coefficients (*r*² > 0.95) (Table 5), which was in agreement with previous reports.^{41,42}

Table 6. Stepwise Regression Model for Predicting Cr Concentration (Y) in Polished Rice Based on Soil Characteristics (n = 32)

stepwise regression models	R ²	F value ^a	T value ^a of the partial regression coefficient	R ² of the partial regression coefficient
Y = 0.561 + 0.0313Cr _T + 0.323Cr _{Ext} + 0.0495Cr(VI) - 0.0116OM - 0.0559Fe(II) - 0.0267clay content	0.965	116.5*		
		Cr _T ^b	7.45**	0.830
		Cr _{Ext} ^c	5.68*	0.718
		Cr(VI)	4.15*	0.673
		OM ^d	2.97*	0.407
		Fe(II)	3.56*	0.656
		Clay	3.12*	0.588

^aSuperscript * and ** indicate significant level at 0.05 and 0.01, respectively. ^bCr_T refers to total Cr. ^cCr_{Ext} refers to Mehlich-3 extractable Cr. ^dOM, organic matter.

The uptake of Cr is affected by soil physical, chemical, and biological properties; therefore, high variability of Cr phytoavailability is often reported in the literature.⁵ With this consideration, soil pH, OM, CEC, Fe(II), Mn(ER), PSD, total soil Cr, Mehlich-3 extractable Cr, and Cr(VI) concentration were integrated together to simulate the combined effects of rhizosphere microenvironment on Cr phytoavailability to polished rice using stepwise regression analysis, and six independent variables "total Cr, Mehlich-3 extractable Cr, Cr(VI), OM, Fe(II), and clay content" were extracted as being significant (Table 6). Both coefficients of multiple correlation and partial regression reached at least the 0.05 statistically significant level. For the multiple linear regressions, the R² values can be used to explain the variation of the dependents.⁵ Table 6 shows that the R² value was above 0.96, which means that more than 96% of variation in Cr concentration of polished rice can be attributed to soil total Cr, Mehlich-3 extractable Cr, Cr(VI), OM, Fe(II), and clay content.

The coefficients of each influence factor can be used to indicate the influence ability of these factors.⁵ Table 6 lists the model parameters of each influence factor. For example, the recommended model suggested that Cr concentration in polished rice was enhanced by higher soil total Cr, higher Mehlich-3 extractable Cr, higher Cr(VI), lower OM, lower Fe(II), and lower clay content (positive coefficients showed a positive effect and vice versa). The contribution of total Cr concentrations in soils indicated that some soil fractions of Cr could also be absorbed indirectly through decomposition of Cr-organic matter complexes by microorganisms.⁴³ Mehlich-3 extractable Cr fractions are assumed to be easily absorbed fractions by plants,⁴² and Cr(VI) exists as highly soluble oxyanionic species in soil.¹⁴ Variables "OM, Fe(II), and clay fraction" could lower soil Cr extractability by promoting Cr(VI) reduction; therefore, these three variables gave the negative effect on Cr phytoavailability. Zeng et al.⁴⁴ also noted that soil properties (e.g., OM and pH) affect Cr availability and its uptake by rice plants. Therefore, such influences should thus be considered in the evaluation of Cr phytoavailability.

Among the six parameters involved in this model, interactions among them were obvious, e.g., Cr concentration in the extractable fraction and Cr(VI) concentration were both correlated with soil total Cr concentration, and also correlated with soil OM, Fe(II), and clay content (Table 3). Furthermore, the coefficients obtained in this model can regulate these cross-effects and make a better model fitting. For example, although there was correlation among the soil total Cr, Mehlich-3 extractable Cr, and Cr(VI) and all three factors had a positive effect on Cr phytoavailability, the Mehlich-3 extractable Cr was the leading influence factor on the Cr phytoavailability to rice

(coefficient of Mehlich-3 extractable Cr was positive and greater than that of the other two parameters). However, this influence is not sufficient, and it is necessary that the total Cr concentration, Cr(VI) concentration, OM, Fe(II), and clay content are to be complementary. This result concurs with the finding of Wang et al.,⁵ who developed an empirical model to correlate Cr phytoavailability to celery and cole with common soil properties and Cr concentrations in soils and also found that soil extractable Cr is the leading influence factor on Cr phytoavailability, with the total Cr concentration, OM, pH, and CEC to complement.

Cr contents ranged from 22 to 500 mg kg⁻¹ with an average of 150 mg kg⁻¹ for most Cr-contaminated soils.⁴⁵ Cr levels (BV, 100, 200, 300, 400 mg kg⁻¹) used in this study represented unpolluted, lightly polluted, and moderately polluted soil. Han et al.⁴⁶ also used the concentrations of 50, 100, 250, and 500 Cr(VI) mg kg⁻¹ to evaluate the phytoavailability and toxicity of Cr(III) and Cr(VI) to *Brassica juncea*. Therefore, the Cr levels used in this study are realistic, comparable to those applied in other contamination studies, and the results should be applicable to field conditions.

Soil Cr Thresholds for Potential Dietary Toxicity in Rice. To ensure environmental and food safety, an effort was made to develop guidelines for acceptable concentrations of potentially harmful Cr in agricultural soils. In this regard, one parameter needs to be considered: the concentration of Cr in polished rice above which food safety for humans is negatively affected. Since Cr phytoavailability differed among soil types, the focus was on development of soil Cr thresholds for representative Chinese soils based on food safety.

According to the estimated safe and adequate daily dietary intake (ESADDI) of Cr proposed by the U.S. National Academy of Sciences, the maximum daily dietary intake of total Cr for adults (based on body weight) was 200 μg.¹⁵ Daily dietary intake (DDI) was determined by the following equation⁴⁷

$$DDI = C_{\text{chromium}} \times D_{\text{food intake}}$$

where C_{chromium} and $D_{\text{food intake}}$ represent the Cr concentration in polished rice (μg g⁻¹) and daily intake of polished rice (g), respectively. The average daily intake of rice for adults was considered to be 323 g person⁻¹ day⁻¹.⁴⁸ According to the above equation of DDI, the calculated maximum level of Cr for polished rice was 0.619 μg g⁻¹ on a dry-weight basis.

Soil Cr thresholds for potential dietary toxicity in rice were calculated according to the maximum level of 0.619 μg g⁻¹ for polished rice and the regression equation in Table 5, and are shown in Table 7. Chromium concentrations in polished rice were significantly related to total Cr, Mehlich 3-extractable Cr, and Cr(VI) concentration in soils, with r^2 values of 0.882–0.973,

Table 7. Soil Cr Thresholds for Potential Dietary Toxicity in Polished Rice Calculated from the Estimated Safe and Adequate Daily Dietary Intake of 200 μg and Regression Equations in Table 5 (mg kg^{-1})

	total Cr	Mehlich-3 extractable Cr	Cr(VI)
Periudic Argosols	56.6	1.54	2.26
Udic Ferrisols	90.6	0.56	0.82
Calcaric Regosols	80.8	0.26	0.68
Stagnic Anthrosols	81.1	0.34	0.84
Mollisols	78.2	0.42	1.32
Ustic Cambosols	51.8	2.18	2.72

0.963–0.993, and 0.874–0.995, respectively. The total Cr thresholds for potential dietary toxicity conformed to the order of Udic Ferrisols > Stagnic Anthrosols > Calcaric Regosols > Mollisols > Periudic Argosols > Ustic Cambosols and were 90.6, 81.1, 80.8, 78.2, 56.6, and 51.8 mg kg^{-1} , respectively. Total Cr thresholds decreased in an order that is similar to the order of Cr(VI) reduction rates; therefore, it is speculated that the high total Cr thresholds in some soils might be attributed to fast rates of Cr(VI) reduction. From this study, Cr concentrations in polished rice were best related to Mehlich-3 extractable Cr concentration in Periudic Argosols, Udic Ferrisols, Mollisols, and Ustic Cambosols, with Mehlich-3 extractable Cr thresholds of 1.54, 0.56, 0.42, and 2.18 mg kg^{-1} , respectively, while the Cr concentrations of polished rice were best related to Cr(VI) concentration in Calcaric Regosols and Stagnic Anthrosols, with Cr(VI) thresholds of 0.68 and 0.84 mg kg^{-1} , respectively. On the basis of the wide range of applicability and simplicity of extraction method, it is proposed that Mehlich-3 extractable Cr is more adequate as Cr thresholds for potential dietary toxicity in rice. This finding was similar to the conclusion of Bhattacharyya et al.,⁴⁹ who noted that the water-soluble and exchangeable Cr was a good soil test index for Cr phytoavailability in rice. Kabata-Pendias⁴² also reported that Mehlich-3 extractable metals, such as soluble, exchangeable, and loosely adsorbed metals, are quite labile and readily available for plants.

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Notes

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