JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Prediction Model for Cadmium Transfer from Soil to Carrot (*Daucus carota* L.) and Its Application To Derive Soil Thresholds for Food Safety

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ABSTRACT: At present, soil quality standards used for agriculture do not fully consider the influence of soil properties on cadmium (Cd) uptake by crops. This study aimed to develop prediction models for Cd transfer from a wide range of Chinese soils to carrot (*Daucus carota* L.) using soil properties and the total or available soil Cd content. Path analysis showed soil pH and organic carbon (OC) content were the two most significant properties exhibiting direct effects on Cd uptake factor (ratio of Cd concentration in carrot to that in soil). Stepwise multiple linear regression analysis also showed that total soil Cd, pH, and OC were significant variables contributing to carrot Cd concentration, explaining 90% of the variance across the 21 soils. Soil thresholds for carrot (cultivar New Kuroda) cropping based on added or total Cd were then derived from the food safety standard and were presented as continuous or scenario criteria.

KEYWORDS: *Daucus carota, cadmium, transfer characteristics, prediction models, soil thresholds*

INTRODUCTION

Because of its high rates of soil−plant transfer, cadmium (Cd) is a contaminant found in most human foodstuffs, which renders diet a primary source of exposure among nonsmoking, nonoccupationally exposed populations.[1]

Consumption of vegetables is one of the most important pathways by which heavy metals enter the food chain.[2] The availability of Cd to vegetable plants varies significantly with soil type due to differences in soil properties. Soil properties exhibiting noticeable effects on the mobility and availability of Cd in soil include pH, organic carbon (OC) content, cation exchange capacity (CEC), texture, Fe, Al, and Mn oxides, and calcium carbonate.[3−5] These soil properties are often intercorrelated, which makes it difficult to determine how each component contributes to Cd uptake from soils. Therefore, simple correlation analysis alone may not be sufficient for establishing a causal relationship between Cd uptake and soil properties. As a means of partitioning correlations into direct and indirect effects and distinguishing between correlation and causation, path analysis has been applied to investigate the relationships between soil properties and the P sorption capacity,[6,7] adsorption of heavy metals,[8] and trace elements concentrations in soils.[9]

At present, most soil quality standards are still based on the total metal content in soil (for example, China and many countries in the European Union) and vary widely across the world.[10] Experiences from the field have given rise to the perception that performing risk evaluations based on the total concentrations alone may lead to an inaccurate assessment of the actual risks.[11] To reduce the apparent contrasts, frameworks for risk assessment and environmental management should consider bioavailability instead of the total metal content only. Bioavailability of metals like Cd depends on soil properties like pH, OC content, CEC, etc. The key for improving soil quality guidelines, therefore, is to develop predictive models that take into account the substantial influence of soil type on the availability and transfer of Cd from soil into crops.[12] In addition, models based on the available fraction of Cd measured by dilute salt extracts (e.g., 0.01 M $CaCl_2$) were also reported to be able to predict Cd levels in arable crops such as rice grains.[13] Previous and contemporary investigations have centered on the soil−plant transfer characteristics of Cd. However, the soil types and sampling areas in those studies are generally limited to small variation ranges, thus the identified influential factors and prediction relationship might lack broader representativeness. Despite the fact that soil−plant transfer models of Cd have been derived for various crops, only a few are actually used to derive national or local soil quality standards. Existing applications include the derivation of soil standards for wheat in The Netherlands and Australia,[14,15] as well as for rice in Taiwan.[10] Applications, however, for carrot (*Daucus carota* L.), which is one of the major consumed vegetables in the world, are still lacking.

China has a wide range of soils developed in different climatic zones, such as acidic red soils in the subtropical region

Received: July 6, 2013
Revised: September 27, 2013
Accepted: September 30, 2013
Published: September 30, 2013

dx.doi.org/10.1021/jf4029859 | *J. Agric. Food Chem.* 2013, 61, 10273−10282

and calcareous soils under arid climates in western and northern regions. It is therefore necessary to develop models of Cd transfer for a wide range of soil environments. The aims of the present study were therefore as follows: (1) to investigate the transfer characteristics of Cd (exogenous Cd salts) from a wide range of Chinese soils to the edible part of the carrot, (2) to identify the major controlling factors and develop prediction models, and then (3) to derive soil thresholds for carrot cropping based on food safety standard.

■ MATERIALS AND METHODS

Soil Description and Experimental Design. Twenty-one soils covering a wide range of soil properties were collected from throughout China (Figure 1). All soils were sampled from the surface (0−20 cm topsoil) of farmlands for pot experiment. The soil was air-dried, homogenized, and passed through a 2 mm sieve prior to use. Selected physical and chemical properties of the soils are shown in Table 1.

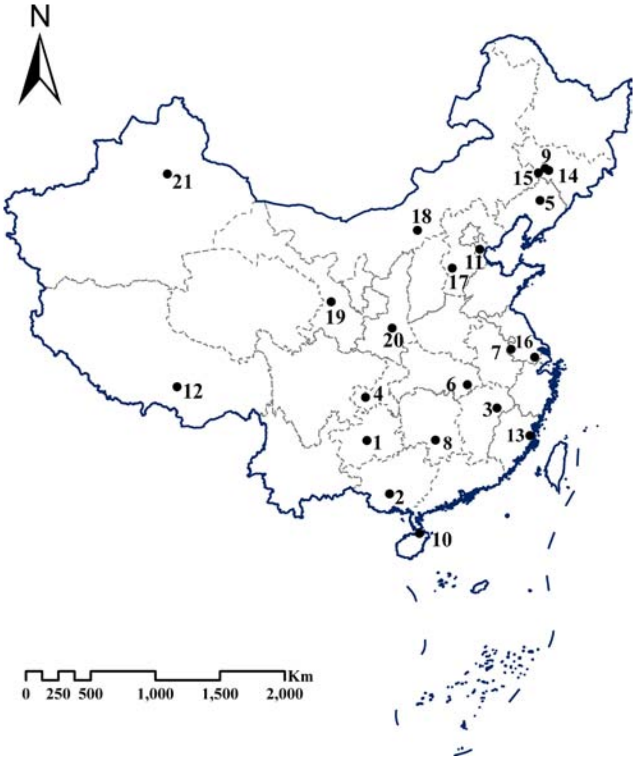

Figure 1. The location of the soil sampling sites.

A greenhouse experiment was conducted in the Institute of Soil Science, Chinese Academy of Sciences, Jiangsu Province, China. Soil samples (7 kg) were placed in each plastic pot (30 cm in upper diameter and 26 cm in height) after mixing thoroughly with an appropriate amount of Cd ($3CdSO_4 \cdot 8H_2O$ solution) on May 25, 2011. Three treatments were applied, including the control (CK, no Cd added to soil), low-Cd (Cd1, 0.3 mg kg^{-1} for soils pH < 7.5 and 0.6 mg kg^{-1} for soils pH > 7.5), and high-Cd (Cd2, 0.6 mg kg^{-1} for soils pH < 7.5 and 1.2 mg kg^{-1} for soils pH > 7.5), according to the Cd limit of the second grade soil (the highest allowable soil Cd concentration for vegetable production) of the National Soil Environmental Quality Standard of China (GB 15618-1995). The soil was then left to equilibrate for about three months. This period is long enough to allow natural equilibration of the various sorptions in the soil.[16] During the equilibration, soil water was maintained at 80% maximum water holding capacity by adding water and weighing the pots on a weekly basis. These pots were arranged in a randomized complete block design with three replicates.

To ensure normal growth and development of plants and to exclude the possible influence of nutrient deficiency, base fertilizers were applied and mixed thoroughly with soil after the equilibration. The doses of N (in urea), P (in $Ca(H_2PO_4)_2$), and K (in K_2SO_4) were equal for all treatments: 0.15 g N, 0.05 g P, and 0.10 g K kg^{-1} soil.

Seeds of carrot cultivar (cv.) New Kuroda, known to be the most widely used for eaten fresh and processing for export in China,[17] were sown directly to the soil in late August 2011. Following emergence, the number of seedlings was thinned to three per pot. The plants were watered to maintain moderate soil moisture during the growing period.

Soil and Plant Analysis. After reaching maturity, carrot was harvested in late December 2011. At harvest, the edible part of the carrot was first washed with tap water, then scrubbed gently using a nylon brush in deionized water to remove adhering soil, and finally rinsed thoroughly with ultrapure water obtained from Milli-Q system (Millipore Corp., USA). Fresh samples were homogenized using a Retsch-grinder (GM 200, Germany). Subsamples of the edible part were digested with HNO_3/H_2O_2 (4:3) in high pressure sealed digestion vessels according to Determination of Cadmium in Foods, National Food Safety Standard of China (GB/T 5009.15-2003). After the equilibration period, soil samples were collected, air-dried, and ground to pass through a 0.149 mm sieve for total Cd analysis.[18] The available Cd extracted with 0.01 M $CaCl_2$[19] in soil samples at harvest was also determined.

Soil pH and electrical conductivity (EC) was measured using a 1:2.5 and 1:5 soil-to-water ratio, respectively. The contents of soil organic carbon (K_2CrO_4−H_2SO_4 oil-bath-heating), cation exchange capacity (1 M ammonium acetate leaching method at pH 7.0), and contents of clay (hydrometer method), calcium carbonate (gasometer flask), and free Fe, Mn, and Al oxides (extracted by dithionite-citrate-bicarbonicum) were analyzed according to the routine analytical methods of agricultural chemistry in soil.[18] The Cd concentration in soil and plants was determined by an atomic absorption spectrophotometer (AAS, Hitachi Z-8000). A plant certified reference material, carrot material (GBW10047, National Research Center for Certified Reference Materials, China), and soil certified reference material (GBW07450, National Research Center for Certified Reference Materials, China) were used to ensure the precision of the analytical procedure. The recovery ratios of the reference carrot and soil ranged from 95% to 106% and 94% to 103%, respectively, throughout the analysis procedure. All chemical reagents used in the Cd analysis were of Guaranteed Reagent (GR) grade, and those used in the analysis of other soil properties were of Analytical Reagent (AR) grade.

Data Analysis. The uptake factor (UF) (also termed bioconcentration factor, transfer factor, enrichment factor) is used to evaluate the transfer potential of a metal from soil to plant. It is traditionally defined as the ratio of metal concentration in plant to the total metal concentration in soil.[20,21] In order to eliminate the potential effects of Cd accumulation in plants caused by Cd background concentration in the soil, on the basis of UF_{total},

$$UF_{total} = \frac{Cd_{carrot}}{Cd_{soil}} \tag{1}$$

we defined UF_{added} as

$$UF_{added} = \frac{Cd_{carrot} - Cd_{carrot\text{-}CK}}{Cd_{soil} - Cd_{soil\text{-}CK}} \tag{2}$$

where Cd_{carrot} and Cd_{soil} are Cd concentration in the edible part of carrot and the experimental soil (including treatment CK, Cd1, and Cd2), respectively, $Cd_{carrot\text{-}CK}$ is Cd concentration in the edible part of carrot grown in control soil, and $Cd_{soil\text{-}CK}$ is Cd concentration in control soil.

Stepwise multiple linear regression (MLR) was used to derive empirical models capable of predicting Cd content in carrot based on soil properties. Soil properties that were not statistically significant (P > 0.05) were eliminated from the multiple regression equation. Soil pH is well correlated with clay content ($r = 0.788$, $P < 0.001$, $n = 21$). Since most of the current soil quality standards are divided by soil pH,

Table 1. Selected Properties of the Soils Used in This Study

soil[a]	location	pH	OC[b] (g kg^{-1})	CEC[c] (cmol kg^{-1})	clay (<0.002 mm, %)	EC[d] (μS cm^{-1})	$CaCO_3$ (g kg^{-1})	Fe_{OX}[e] (g kg^{-1})	Mn_{OX}[f] (g kg^{-1})	Al_{OX}[g] (g kg^{-1})	background Cd (mg kg^{-1})
1	Guiyang, Guizhou	4.67	20.6	15.4	55.8	119	h	60.2	0.27	103.2	0.16
2	Nanning, Guangxi	4.81	14.6	7.63	36.7	105	h	38.3	0.06	61.1	0.12
3	Yingtan, Jiangxi	4.84	5.43	9.31	45.8	52.7	h	35.1	0.18	69.6	0.12
4	Chongqing	4.99	9.92	16.9	20.2	78.6	h	30.6	0.58	73.2	0.34
5	Shenyang, Liaoning	5.35	8.81	15.8	22.4	136	h	26.5	0.66	70.1	0.22
6	Daye, Hubei	5.68	10.1	12.3	29.6	73.4	h	66.9	0.56	90.2	0.58
7	Nanjing, Jiangsu	6.28	12.9	12.1	15.9	197	h	19.5	0.21	48.1	0.22
8	Qiyang, Hunan	6.31	16.5	14.0	31.3	90.2	h	32.8	0.56	65.0	0.50
9	Gongzhuling, Jilin	6.52	14.0	24.9	33.6	246	h	20.8	0.91	65.0	0.21
10	Haikou, Hainan	6.83	6.06	4.53	17.8	35.5	h	32.1	0.31	46.1	0.06
11	Tianjin	6.93	9.90	24.1	36.5	237	h	28.1	0.74	73.7	0.19
12	Lhasa, Xizang	7.01	12.1	8.53	10.1	77.9	h	25.6	0.60	63.4	0.06
13	Fuzhou, Fujian	7.12	9.32	10.2	20.9	127	h	27.8	0.24	108	0.12
14	Gongzhuling, Jilin	7.30	15.2	23.2	33.4	150	h	23.2	0.57	68.0	0.12
15	Shuangliao, Jilin	7.88	21.6	14.4	6.35	286	137	15.5	0.48	50.2	0.23
16	Suzhou, Jiangsu	8.04	5.55	8.18	13.8	206	42.4	30.6	0.69	61.9	0.21
17	Shijiazhuang, Hebei	8.23	8.58	9.16	9.16	106	20.7	22.5	0.66	64.4	0.16
18	Hohhot, Inner Mongolia	8.37	9.54	9.70	8.34	80.0	5.45	19.7	0.59	65.1	0.14
19	Lanzhou, Gansu	8.41	7.38	7.63	11.3	252	123	29.6	0.70	56.9	0.29
20	Xi'an, Shaanxi	8.65	5.88	6.86	8.21	97.8	102	23.8	0.65	56.6	0.12
21	Urumqi, Xinjiang	8.67	4.30	6.65	10.3	222	55.7	22.5	0.70	60.6	0.23

[a] Soil numbers were sequenced in the order of increasing pH. [b] Organic carbon. [c] Cation exchange capacity (buffered). [d] Electrical conductivity. [e] Free Fe oxide. [f] Free Mn oxide. [g] Free Al oxide. [h] Not detectable (<5 g kg^{-1}).

which is more readily available, clay was not included as an independent variable in the regression analysis to avoid issues of collinearity.[22] A path analysis (PA) model was applied to examine the causal path of soil properties to Cd uptake factor (Figure 2). PA was performed for UF_{total} and UF_{added} separately. The direct effects of soil properties on UF are represented by single-headed arrows, while correlation coefficients between soil properties are represented by double-headed arrows. Direct and indirect effects in the PA are derived from (i) MLR of soil properties on UF and (ii) simple correlation coefficients between soil properties. The direct effects of soil properties on UF are termed *path coefficients* and are standardized partial regression coefficients for each of the soil properties in the MLR against UF.[8] Indirect effects of soil properties on UF were determined from the product of the simple correlation coefficient between soil properties and the path coefficient (i.e., one double-headed arrow and one single-headed arrow).[23] The correlation between UF and a soil property is the sum of the direct and indirect coefficients, as described by

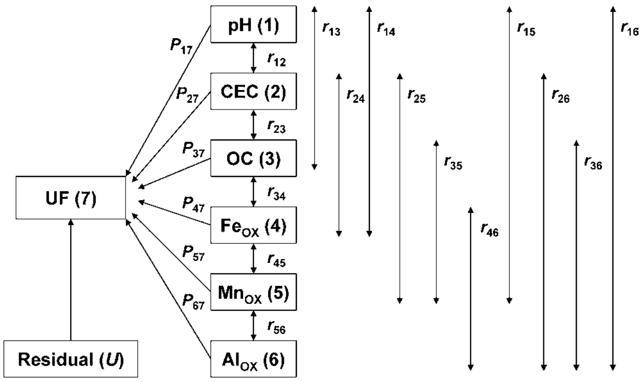

Figure 2. Path analysis diagram for the relationship between Cd uptake factor (UF) and soil properties. Single-headed arrows represent the direct effects (P_{ij}) of soil properties on UF, while double-headed arrows represent the simple correlation coefficients (r_{ij}) of soil properties. Subscript designations are as follows: 1, soil pH; 2, cation exchange capacity (CEC); 3, organic carbon (OC); 4, free Fe oxide (Fe_{OX}); 5, free Mn oxide (Mn_{OX}); 6, free Al oxide (Al_{OX}); 7, Cd uptake factor (UF).

$$r_{17} = P_{17} + r_{12}P_{27} + r_{13}P_{37} + r_{14}P_{47} + r_{15}P_{57} + r_{16}P_{67} \quad (3)$$

$$r_{27} = r_{12}P_{17} + P_{27} + r_{23}P_{37} + r_{24}P_{47} + r_{25}P_{57} + r_{26}P_{67} \quad (4)$$

$$r_{37} = r_{13}P_{17} + r_{23}P_{27} + P_{37} + r_{34}P_{47} + r_{35}P_{57} + r_{36}P_{67} \quad (5)$$

$$r_{47} = r_{14}P_{17} + r_{24}P_{27} + r_{34}P_{37} + P_{47} + r_{45}P_{57} + r_{46}P_{67} \quad (6)$$

$$r_{57} = r_{15}P_{17} + r_{25}P_{27} + r_{35}P_{37} + r_{45}P_{47} + P_{57} + r_{56}P_{67} \quad (7)$$

$$r_{67} = r_{16}P_{17} + r_{26}P_{27} + r_{36}P_{37} + r_{46}P_{47} + r_{56}P_{57} + P_{67} \quad (8)$$

where r_{ij} is the simple correlation coefficient between UF and a soil property, P_{ij} is the path coefficient between UF and a soil property, and $r_{ij}P_{ij}$ is the indirect effect of a soil property on UF.

In addition, an uncorrelated residue (U) that represents the unexplained part of an observed variable in the path model was calculated using the following equation:

$$U = \sqrt{1 - R^2} \quad (9)$$

where R^2 is the coefficient of determination of the multiple regression equation between UF and the six soil properties.

Data were analyzed with statistical package SPSS 18.0 and SigmaPlot 11.0. All data were log-transformed (except for pH) prior to analysis due to their non-normal distributions.

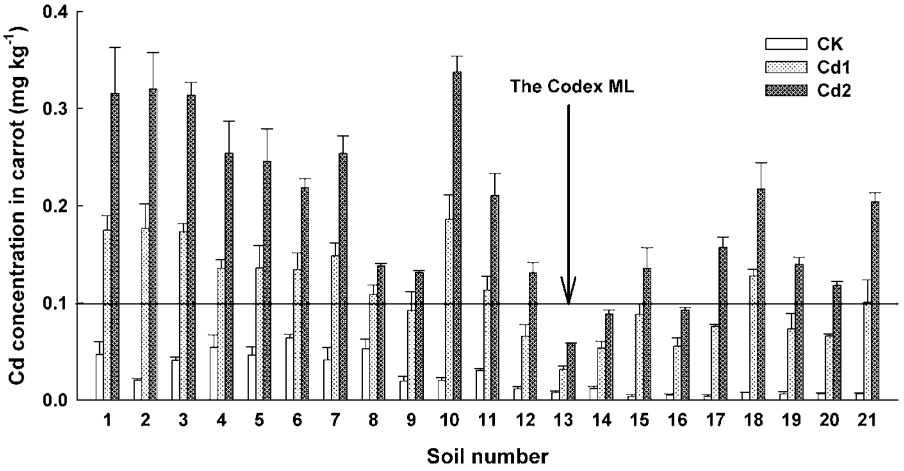

Figure 3. Cd concentration (mg kg^{-1}, FW) in the edible part of the carrot under different Cd treatments (CK, the control; Cd1, low-Cd treatment; Cd2, high-Cd treatment). The Codex maximum level (ML) is 0.1 mg kg^{-1} in root and tuber vegetables recommended by the Codex Alimentarius Commission of FAO and WHO.

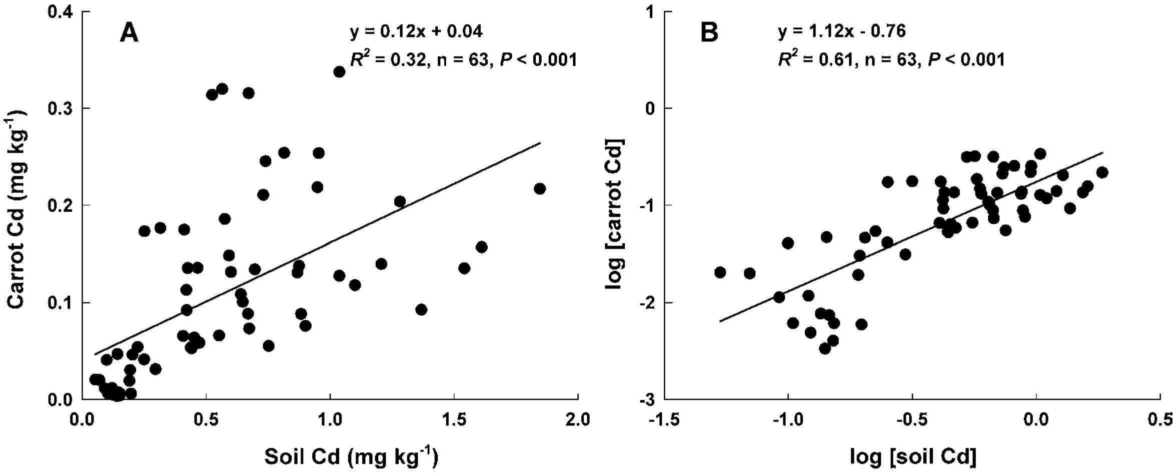

Figure 4. Relationships between Cd concentration in carrot and soil in normal (A) and log-transformed (B) formats.

■ RESULTS

Effects of Different Soil Types on Cd Transfer from Soil to Carrot. According to the Codex General Standard for Contaminants and Toxins in Food and Feed recommended by the Codex Alimentarius Commission of FAO and WHO,[24] the Codex maximum level (ML) in root and tuber vegetables for Cd is 0.1 mg kg^{-1} fresh weight.

To compare our results to the Codex ML, Figure 3 shows the Cd concentration in the edible part of the carrot expressed on a fresh weight basis. Under low Cd treatment, Cd concentration in carrot ranged from 0.03 to 0.19 mg kg^{-1} (average 0.11 mg kg^{-1}) for the 21 soils, with all of the soils with pH < 6.5 and only four of the soils with pH > 6.5 (soils 10, 11, 18, and 21) exceeding the Codex ML (0.1 mg kg^{-1}). Under high Cd treatment, Cd concentration in carrot ranged from 0.06 to 0.34 mg kg^{-1} (average 0.19 mg kg^{-1}) for the 21 soils, with soils 13, 14, and 16 not exceeding the Codex ML.

A significant and positive correlation ($R^2 = 0.32$, $P < 0.001$) was obtained between Cd concentration in carrot and that in soil, as shown in Figure 4A. The log-transformed data provided much better correlation ($R^2 = 0.61$, $P < 0.001$, Figure 4B) due to assurance of variance homogeneity[25] and the linear relationship provided by the log-transformed Freundlich-type functions as shown in eq 10.

As shown in Figure 5A, UF_{total} with Cd additions increased substantially on different soils compared with control due to the higher bioavailability of added Cd. Generally, the bioavailability of added metal salts to soils is higher than the indigenous metals in soils. Negligible difference was observed for UF_{total} between low and high Cd treatments for most of the soils with low Cd background concentration. Under control and the two Cd treatments, the three maxima of UF_{total} were all observed in the most acidic soils 3, 2, and 1 (pH 4.84, 4.81, and 4.67) with a decreasing OC content. The minimum of UF_{total} (0.02) in control soil was found in soil 15, with the highest OC, EC, and $CaCO_3$ content. Under low Cd treatment, the three minima of UF_{total} (0.07, 0.08, and 0.10) were found in soils 16, 17, and 15. Insignificant differences for UF_{total} were observed among soils 8, 12, and 21 and among soils 13, 14, 15, 18, 19, and 20. Noticeably, no significant difference was found between soil 8 and 21 with a discrepancy in pH of 2.36. This was probably due to the discrepancy of OC content (16.5 and 4.30 g kg^{-1}) between the two soils. Under high Cd treatment, soils 16, 15, and 17 still exhibited the minima of UF_{total} (0.07, 0.09, and 0.10).

As for UF_{added}, similarity between low and high Cd treatments was observed, and UF_{added} in soil 3 was still the highest and soil 16 the lowest among all soils (Figure 5B).

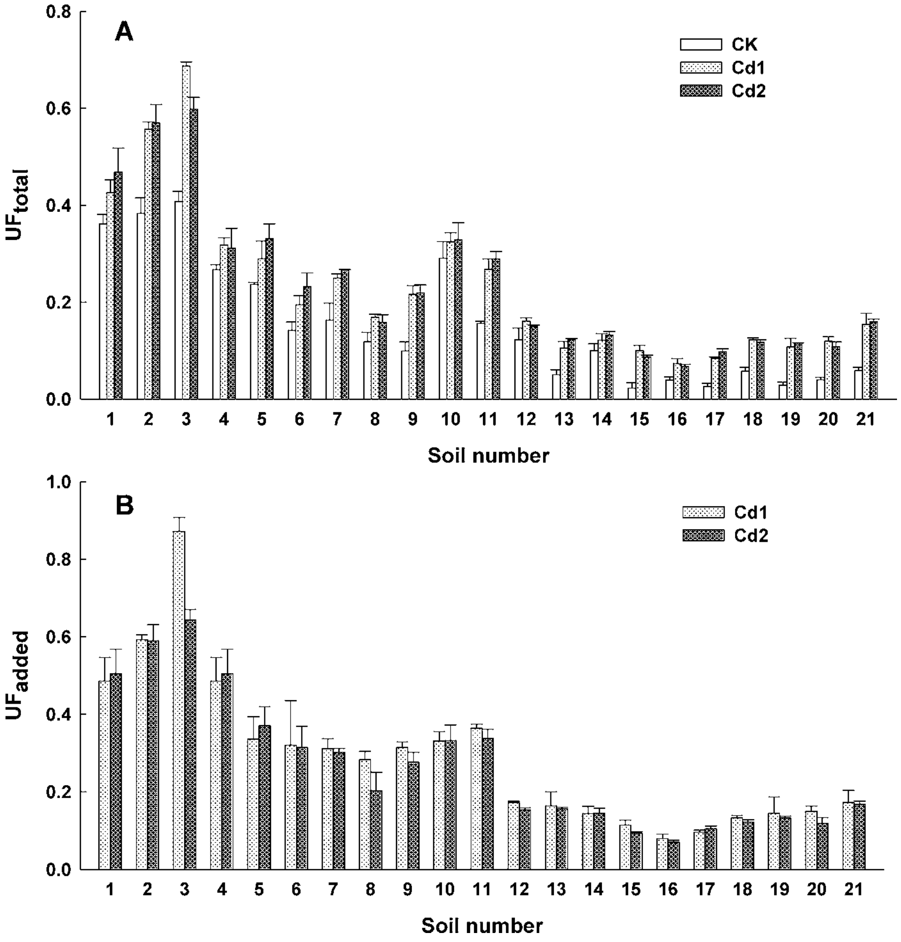

Figure 5. UF_{total} (A) and UF_{added} (B) of carrot in the 21 soils under different Cd treatments (CK, the control; Cd1, low-Cd treatment; Cd2, high-Cd treatment).

Table 2. Direct Effects (Diagonal, Italics) and Indirect Effects (off Diagonal) of Soil Properties on Cd Uptake Factor[a]

variable	pH	CEC	OC	Fe_{OX}	Mn_{OX}	Al_{OX}	r	R^2	U
					UF_{total}				
pH	*−0.82*[b]	−0.06	0.11	−0.05	−0.13	0.09	−0.86[b]	0.84[b]	0.55
CEC	0.27	*0.19*	−0.19	−0.00	−0.07	−0.09	0.11		
OC	0.30	0.12	*−0.31*[c]	0.00	0.04	−0.04	0.11		
Fe_{OX}	0.50	−0.01	−0.01	*0.07*	0.07	−0.14	0.49[c]		
Mn_{OX}	−0.45	0.05	0.06	−0.02	*−0.24*	0.01	−0.59[b]		
Al_{OX}	0.32	0.07	−0.06	0.04	0.01	*−0.23*[d]	0.16		
					UF_{added}				
pH	*−0.84*[b]	−0.08	0.12	−0.06	−0.10	0.07	−0.89[b]	0.85[b]	0.52
CEC	0.27	*0.25*	−0.20	−0.01	−0.05	−0.07	0.20		
OC	0.31	0.15	*−0.33*[c]	0.00	0.03	−0.03	0.14		
Fe_{OX}	0.51	−0.02	−0.01	*0.10*	0.06	−0.11	0.53[c]		
Mn_{OX}	−0.46	0.07	0.06	−0.03	*−0.18*	0.01	−0.53[c]		
Al_{OX}	0.33	0.10	−0.06	0.06	0.01	*−0.19*	0.24		

[a] UF_{total}, uptake factor based on total soil Cd; UF_{added}, uptake factor based on added soil Cd; CEC, cation exchange capacity; OC, organic carbon; Fe_{OX}, free Fe oxide; Mn_{OX}, free Mn oxide; Al_{OX}, free Al oxide. [b] Significant at the level $P < 0.001$. [c] Significant at the level $P < 0.01$. [d] Significant at the level $P < 0.05$.

Nevertheless, the elimination of background Cd concentration in the calculating formula resulted in increased Cd transfer factor for each soil compared with UF_{total}.

Major Factors Affecting Cd Uptake from Different Soils. Path analysis (PA) was applied to partition the direct and indirect effects of soil properties on Cd uptake factor. The uncorrelated residual values (U) were 0.55 and 0.52, and the coefficients of determination (R^2) were 0.84 and 0.85, indicating that the PA explained 84% and 85% of variation in UF_{total} and UF_{added} respectively (Table 2). PA partitioned each r value into one direct effect and five indirect effects. Soil pH ($P_{17} = -0.82$, $P < 0.001$), OC ($P_{37} = -0.31$, $P < 0.01$), and Al_{OX} ($P_{67} = -0.23$, $P < 0.05$) had significant direct effects on UF_{total}, while only soil pH ($P_{17} = -0.84$, $P < 0.001$) and OC ($P_{37} = -0.33$, P

< 0.01) had significant direct effects on UF_{added}. The simple correlation between OC and UF_{total} (or UF_{added}) was not significant. Furthermore, PA revealed that the significant correlations between Fe_{OX} and UF_{total} or UF_{added} and between Mn_{OX} and UF_{total} or UF_{added} were mainly due to the indirect effects of soil pH. These results further demonstrated that simple correlation analysis was not enough in describing the relationship between Cd uptake and soil properties.

Prediction Models for Cd Transfer from Soil to Carrot. For metal transfer from soil to plant, Freundlich-type functions are often used:[26,27]

$$C_{plant} = 10^{a} C_{soil}{}^{b} \quad \text{or} \quad \log[C_{plant}] = a + b \log[C_{soil}] \tag{10}$$

where C_{plant} is metal concentration in the plant, C_{soil} is metal concentration in the soil, and a and b are constants.

The Freundlich-type equation can be extended, using soil properties like pH, OC, CEC, clay, etc. The log-transformed Freundlich model is commonly applied,[28−30] although the choice of soil properties included in each of the studies varied. In the present study, stepwise multiple linear regression (MLR) was used to derive the extended Freundlich-type equations. Table 3 presents the prediction equations of different Cd sources (control, total Cd, and added Cd under two Cd additions, and the combined data of the three Cd treatments). Cd concentrations in carrot at low and high Cd treatments were pooled here due to a negligible difference of UF_{total} or UF_{added} (as discussed earlier). The results of MLR analysis agreed well with PA and found that aside from the total soil Cd concentration, the combination of pH and OC were the two most important soil properties related to Cd concentration in carrots under low and high Cd treatments. All equations displayed the same trend that carrot Cd concentration was positively related to soil Cd and negatively correlated with soil pH and OC (in control soil, OC was not included in the equation due to its nonsignificance). Noteworthily, the correlation between carrot Cd and soil added Cd was higher than that of soil total Cd. The concept of exogeneity was introduced and applied in the calculation of Cd transfer from soil to carrot in order to exclude the effect of soil background Cd (control Cd) as has been done by Liang et al.[31] The higher correlation between carrot Cd and soil added Cd indicates a better representativeness for characterization of Cd transfer characteristics. Combining the data of the three Cd treatments (63 observations) considerably improved the variance explained from $R^2 = 0.73$ to $R^2 = 0.90$, as indicated in Table 3.

Table 3. Stepwise Multiple Linear Regression Equations

Cd sources[a]	regression equations	n	R^2	P	RMSE
control	$\log[Cd_{carrot}] = 0.51 - 0.26pH + 0.64 \log[Cd_{soil}]$	21	0.85	<0.001	0.16
total	$\log[Cd_{carrot}] = 0.85 - 0.18pH + 0.94 \log[Cd_{soil}] - 0.32 \log[OC]$	42	0.73	<0.001	0.12
added	$\log[Cd_{carrot}] = 0.90 - 0.19pH + 0.74 \log[Cd_{soil}] - 0.26 \log[OC]$	42	0.77	<0.001	0.11
combined	$\log[Cd_{carrot}] = 1.30 - 0.24pH + 1.27 \log[Cd_{soil}] - 0.40 \log[OC]$	63	0.90	<0.001	0.17

[a] There are four different Cd sources: background Cd in control treatment (control), total Cd in two Cd addition treatments (total), added Cd in two Cd addition treatments (added), and the combined data of all treatments (combined).

In most cases, inclusion of soil properties improves the correlation performance between Cd concentration in plant and in soil compared with those based on soil Cd concentration only. In this study, R^2 between $\log[Cd_{carrot}]$ and $\log[Cd_{soil}]$ was 0.61 ($P < 0.001$). Upon combination of soil Cd and pH, the explained variance improved to $R^2 = 0.88$ ($P < 0.001$). Upon further introduction of OC into the equation, the regression coefficient rose to $R^2 = 0.90$ ($P < 0.001$). Other soil factors, like CEC, clay, EC, free Fe, Mn, or Al oxide contents failed to significantly improve the correlation performance and therefore were excluded here.

The accuracy of the prediction models was determined by plotting measured Cd concentration in carrot against the corresponding calculated Cd concentration (Figure 6). The vast majority of the predicted values were within the 95% prediction intervals. The root-mean-square error (RMSE) values were 0.16, 0.12, 0.11, and 0.17 log unit for the models based on control, total Cd, added Cd, and the combined data, respectively (Table 3). Thus, the developed models were reliable predictors of the transfer of Cd from these soils to carrot, and furthermore the model based on added Cd provided the highest predictability (RMSE = 0.11).

Performance of $CaCl_2$ Extractant To Predict Cd Concentration in Carrot. Carrot Cd concentrations were significantly positively correlated with 0.01 M $CaCl_2$ extractable Cd ($R^2 = 0.41$, $P < 0.001$, $n = 63$) under the three Cd treatments, and the log transformation provided further better correlation with $R^2 = 0.57$. No significant correlation ($R^2 = 0.06$, $P = 0.054$, $n = 63$) was observed between the $CaCl_2$ extractable Cd and total soil Cd concentrations. The results of stepwise MLR showed that the $CaCl_2$ extractable Cd could be predicted by total soil Cd, pH, and OC (eq 11). Furthermore, Cd in carrot can be related to the $CaCl_2$ extractable Cd in soil as shown in eq 12:

$$\log[CaCl_2\text{-}Cd] = 1.66 - 0.31pH + 1.04 \log[Cd_{soil}] - 0.70 \log[OC] \quad (R^2 = 0.70, \quad P < 0.001, \quad n = 63, \quad RMSE = 0.32) \tag{11}$$

$$\log[Cd_{carrot}] = 0.70 \log[CaCl_2\text{-}Cd] - 0.13 \quad (R^2 = 0.57, \quad P < 0.001, \quad n = 63, \quad RMSE = 0.35) \tag{12}$$

Derivation of Soil Cd Thresholds for Carrot Cropping. Soil thresholds for added Cd can be back-calculated from the Codex ML (0.1 mg kg^{-1}) and the added Cd regression equation in Table 3. The derived calculation formula based on added Cd was shown as the continuous criteria in Table 4. Scenario criteria could also be calculated for different combinations of soil pH and OC content as shown in Table 4. The added Cd thresholds ranged from 0.12 to 0.86 mg kg^{-1} in soils with pH range 5.5−8.0 and OC range 5−20 g kg^{-1}. In order to compare with the current National Soil Environmental Quality Standard of China (GB 15618-1995), which is based on total Cd, Table 4 also presents the total Cd thresholds with a locally known or default soil Cd background concentration of 0.13 mg kg^{-1} according to the 50th percentile concentration of background Cd concentrations in Chinese agricultural soils from a national soil survey in 1990.[32] The total Cd thresholds for carrot (cv. New Kuroda) cropping dropped from 0.30 to

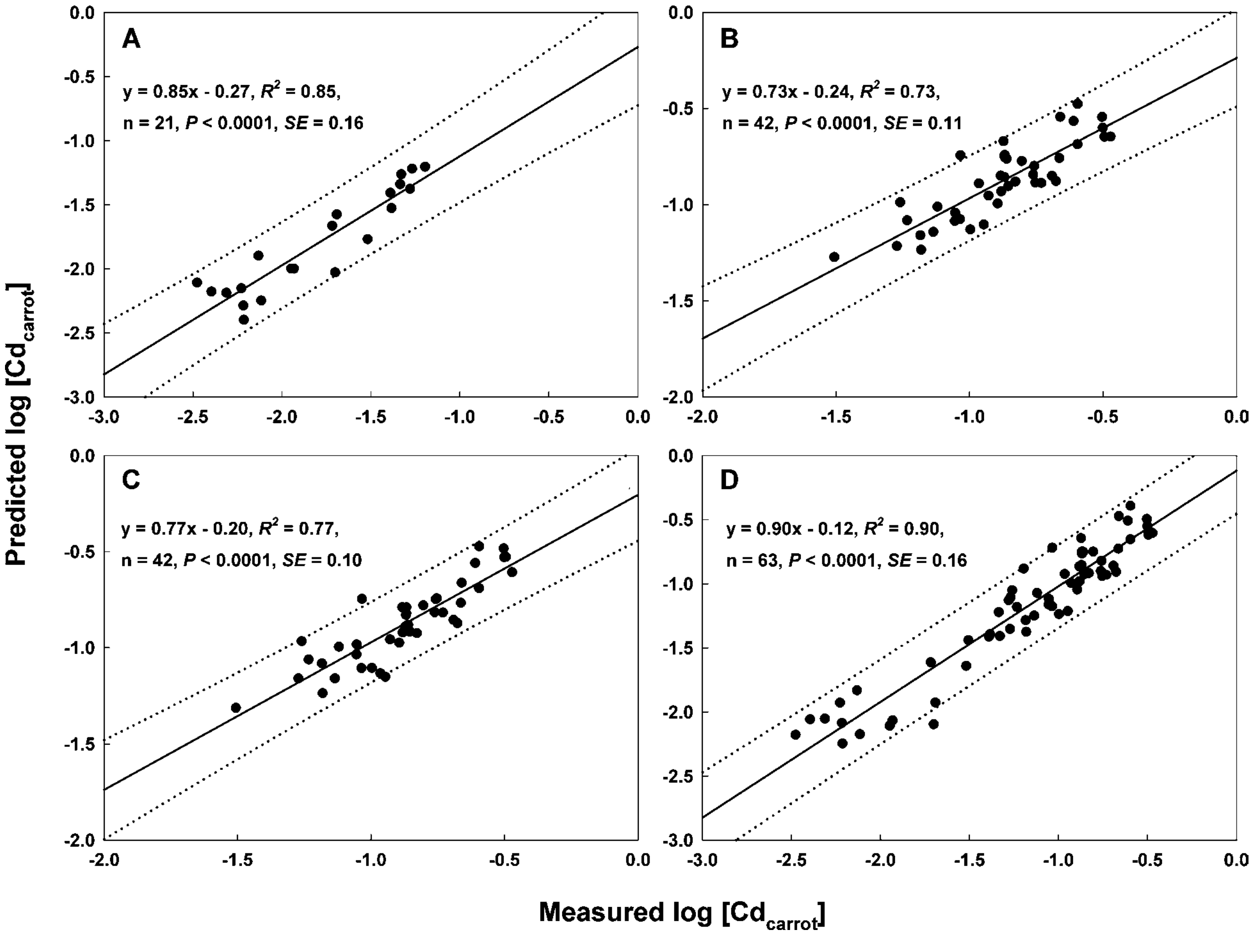

Figure 6. Relationships between measured log[Cd_{carrot}] and predicted log[Cd_{carrot}] of different Cd sources: background Cd in control treatment (A), total Cd in Cd addition treatments (B), added Cd in Cd addition treatments (C), and combined data in all treatments (D). The solid lines indicate regression lines, and the dotted lines indicate 95% prediction intervals.

Table 4. Soil Cd Thresholds for Carrot (cv. New Kuroda) Cropping (mg kg^{-1})

		scenario criteria[a]								
		pH < 6.5			pH 6.5−7.5			pH > 7.5		
approach	continuous criteria	A[b]	B	C	A	B	C	A	B	C
added Cd	$10^{(0.26\text{pH}+0.34\log\text{OC}-2.55)}$	0.12	0.16	0.20	0.30	0.38	0.48	0.54	0.68	0.86
total Cd with known C_b[c]	$10^{(0.26\text{pH}+0.34\log\text{OC}-2.55)}$ + C_b	0.12 + C_b	0.16 + C_b	0.20 + C_b	0.30 + C_b	0.38 + C_b	0.48 + C_b	0.54 + C_b	0.68 + C_b	0.86 + C_b
total Cd with default C_b	$10^{(0.26\text{pH}+0.34\log\text{OC}-2.55)}$ + 0.13	0.25	0.29	0.33	0.43	0.51	0.61	0.67	0.81	0.99
current standard	not available		0.30			0.30			0.60	

[a] The rounded thresholds at soil pH values of 5.5, 7.0, and 8.0 were used for scenarios of soil pH < 6.5, 6.5−7.5, and >7.5, respectively. [b] A, B, and C were scenarios with soil OC content 5, 10, and 20 g kg^{-1}, respectively. [c] C_b is the background concentration of soil Cd, with a default value of 0.13 mg kg^{-1}.[32]

0.25 for acidic soils (pH 5.5) with low OC content (5 g kg^{-1}) and rose from 0.60 to 0.99 for alkaline soils (pH 8.0) with high OC content (20 g kg^{-1}) compared with the current standard.

DISCUSSION

Both multiple regression and path analysis were used to examine the relationship between Cd uptake by carrot and soil properties in the present study. Path analysis confirmed the relationship of the multiple regressions but in some cases allowed us to more specifically identify the soil property most associated with the Cd concentration in carrot.[9] It was observed from the results of PA and stepwise MLR that soil pH and OC reduce Cd uptake by carrot in the present study. Soil pH is often termed the master soil variable because it controls the dissolution and precipitation of metal solid phases, complexation and acid−base reactions of metal species, and metal sorption.[33] The direct effect of pH is its influence on Cd speciation in soil solution. Soil organic matter is a major contributor to the pH-dependent negative charge in soils, which gives rise to the ability to retain cationic metals of soils, resulting in low Cd concentration in soil solution as well as low plant uptake.[34]

To develop scientifically sound strategies for the management of Cd contaminated soils, it is important to estimate the human Cd burden from vegetable consumption. For this purpose, models describing Cd transfer from soil to vegetables have to be established. Both empirical and mechanistic models are currently being developed to describe Cd transfer from soil to crops. Mechanistic models like the free ionic activity model[35] and the biotic ligand model[36] still need to be improved further before application to field. The main limitation of such models is the focus on uptake processes by plant roots while transfer into above ground parts is not yet accounted for. Recently, Francois et al.[37] demonstrated that empirical soil−plant transfer models performed better than mechanistic models to predict the Cd content in wheat. An advantage of Freundlich-type soil−plant transfer relationship is its simplicity and applicability. Most equations use variables that are available from soil investigations, such as total metal content, pH, OC, or CEC. However, these equations should not be used for soils where concentrations of metals are outside the range from which the regressions were derived.[38]

The much higher correlation between carrot Cd concentrations and 0.01 M $CaCl_2$ extractable Cd indicated that 0.01 M $CaCl_2$ provided a better estimate of Cd phytoavailability compared with total soil Cd concentration alone. Nonetheless, the multiple linear models for Cd content in carrot with total soil Cd, pH, and OC as predictors (Table 3) performed better than the model with $CaCl_2$ extractable Cd as a single predictor (eq 12). This is consistent with the findings of previous studies. Brus et al.[29] demonstrated that the multiple linear model for Cd content in rice grains with 0.43 M HNO_3 extractable Cd (the pool of Cd sorbed by soil solid phase), pH, clay, and soil organic matter as predictors performed much better (R_{adj}^2 = 0.66) than the model with $CaCl_2$ extractable Cd as a single predictor (R_{adj}^2 = 0.28). Similarly, Chaudri et al.[39] also found that the stepwise addition of soil pH and OC to soil total Cd resulted in better prediction of wheat grain Cd concentrations (R_{adj}^2 = 0.78), whereas their inclusion with NH_4NO_3 extractable Cd did not improve the relationship any further (R_{adj}^2 = 0.56).

At present, most soil quality standards across the world are still based on the total Cd approach. For example, Brus et al.[14] obtained the critical threshold for total Cd ranging from 0.5 to 1 mg kg^{-1} for wheat grown under temperate climate conditions in Netherlands. McLaughlin et al.[15] calculated the critical soil total Cd concentrations of 0.3 mg kg^{-1} at pH 4.5 in a sandy soil and 1.0 mg kg^{-1} in clay soils at pH 7 for field-grown wheat in Australia. More recently, Römkens et al.[10] derived soil quality standards based on total Cd for both Japonica and Indica-type rice cultivars in Taiwan, with range from less than 0.3 to more than 6 mg kg^{-1}. The data sets used for deriving these standards all come from paired soil and plant samples collected from the field. However, since Cd occurs naturally and is present in the soil at concentrations that can vary quite considerably across different regions owing to the local geological heterogeneity, these early proposed standards were found to be close to or even below the natural background level of soil Cd in some cases. To overcome this problem, the approach of added metal salts to soils for risk assessment was developed.[40] This approach, which has been applied in the present study, has therefore the advantage of better applicability to soils with high background levels of Cd compared with the total Cd approach. In order to compare with the current Chinese soil quality standard, we also calculated the total Cd thresholds with a default soil Cd background concentration of 0.13 mg kg^{-1}. The differences in thresholds for different soil types emphasize the necessity to include soil properties when assessing the suitability of a specific soil for crop production. The results also suggest that the current national soil quality standard is only valid for soils with limited combinations of soil pH and OC content from the point of view of food safety and might have overestimated the associated risk for carrot (cv. New Kuroda) production in soils with pH 6.5−7.5. Similar total Cd thresholds in former studies were obtained in field and pot trials for other rootstalk vegetables. Shentu et al.[41] found the critical soil total Cd concentration for radish was 0.12 and 0.37 mg kg^{-1} in two typical soils of southeastern China. Sun et al.[42] calculated soil total Cd threshold of 0.94 mg kg^{-1} for rootstalk vegetable (carrot, and asparagus lettuce) fields in Guangdong Province, China. These thresholds were all within the range of the calculated total Cd thresholds in our study.

In the present study, both the pH range and OC interval in the 21 soils were wide enough to guarantee the significance of the two variables in the regression models, and particularly variable charge soils with low pH values and highly alkaline soils with high pH values were also involved. It must be noted that these models must be applied only within the boundaries of their calibration.[43] Data on soil properties like pH and OC could be obtained from the database of national soil survey. Therefore, the prediction models in the present study can also be used to derive regional soil quality criteria and region-specific calculations of human exposure to Cd. Using known local soil properties enables local policy makers to make better assessments on the safe use of soil and the production of safe food.

Aside from Cd availability in soil, differences between vegetable cultivars need to be considered when dealing with soil protection of vegetable production fields.[44,45] The comparisons were not incorporated into the derivation of soil thresholds for this study. Therefore, the proposed thresholds could only be applied to the carrot cultivar (New Kuroda) used in this study at present. Validation of the present experiment results to field conditions and adaption for different cultivars still need further investigation.

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Funding

This research was financially supported by the Special Fund for Agro-scientific Research in the Public Interest (Grant No. 200903015) and the GanPo 555 Talents Program of Jiangxi Province, China.

Notes

The authors declare no competing financial interest.

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