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Phytotoxicity and accumulation of chromium in carrot plants and the derivation of soil thresholds for Chinese soils

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ABSTRACT

Soil environmental quality standards in respect of heavy metals for farmlands should be established considering both their effects on crop yield and their accumulation in the edible part. A greenhouse experiment was conducted to investigate the effects of chromium (Cr) on biomass production and Cr accumulation in carrot plants grown in a wide range of soils. The results revealed that carrot yield significantly decreased in 18 of the total 20 soils with Cr addition being the soil environmental quality standard of China. The Cr content of carrot grown in the five soils with pH > 8.0 exceeded the maximum allowable level (0.5 mg kg^{-1}) according to the Chinese General Standard for Contaminants in Foods. The relationship between carrot Cr concentration and soil pH could be well fitted (R^2 = 0.70, P < 0.0001) by a linear–linear segmented regression model. The addition of Cr to soil influenced carrot yield firstly rather than the food quality. The major soil factors controlling Cr phytotoxicity and the prediction models were further identified and developed using path analysis and stepwise multiple linear regression analysis. Soil Cr thresholds for phytotoxicity meanwhile ensuring food safety were then derived on the condition of 10 percent yield reduction.

1. Introduction

Chromium (Cr) is a relatively abundant transition metal in soils, mainly as the trivalent oxidation state of Cr(III) (Allué et al., 2014). Cr(III) is considered a beneficial nutrient in trace amounts for humans and animals, while the hexavalent form, Cr(VI), is regarded as a class A human carcinogen (Han et al., 2004). Cr is a nonessential trace element for plants; its toxicity to plants is observed at multiple levels from reduced yield to effects on leaf and root growth, through the inhibition of enzymatic activities and mutagenesis (Singh et al., 2013).

Soil physicochemical properties, such as pH, E_h (V), cation exchange capacity (CEC), organic carbon and clay content, play critical roles in controlling the speciation, mobility and bioavailability of Cr (Choppala et al., 2013). These properties are often intercorrelated, which makes it difficult to determine how each component contributes to Cr toxicity and accumulation in plants. Therefore, simple correlation analysis alone may be insufficient for establishing a causal relationship between Cr uptake or toxicity and soil properties. As a means of partitioning correlations into direct and indirect effects and distinguishing correlation from causation, path analysis has been applied to investigate the relationships between soil properties and the adsorption of heavy metals and phosphorus (Basta et al., 1993; Ige et al., 2007), trace elements concentrations in soils (Richards et al., 2012), and cadmium (Cd) uptake by carrot plants (Ding et al., 2013).

The use of soil properties to explain the phytotoxicity of metals across different soils is one of the key steps in improving risk assessments of metals in soils (Warne et al., 2008). In the past few years, quantitative relationships between the phytotoxicity of metals such as copper, nickel, and zinc added to a wide range of soils and soil properties have been more extensively studied (Rooney et al., 2006; Warne et al., 2008; Li et al., 2011) than that of Cr (Chen et al., 2008).

Critical loads or criteria of Cr in soils have been developed for environmental protection in many countries. The values for Cr vary widely, mostly in the range of 50 – 600 mg Cr kg^{-1} soil (Ma and Hooda, 2010). The wide variation of soil criteria in different countries is at least partly due to insufficient Cr ecotoxicity data. Soil environmental quality standard for farmland should be established considering both the effects of Cr on the crop growth

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(biomass production) and the Cr accumulation in the edible part (Dai et al., 2009). Currently, there is little information regarding the phytotoxicity and/or accumulation of Cr in crops grown in a wide range of soils. It is still unclear whether phytotoxicity or food quality should be preferably considered when deriving the soil Cr thresholds.

China has a wide range of soils developed in different climatic zones, such as acidic red soils in subtropical China and calcareous soils in western and northern China. Carrot (*Daucus carota* L.) is one of the major consumed vegetables in the world, and it was recommended by the US EPA as a biomarker for toxicity assessment in terrestrial ecosystem (Environmental Protection Agency (EPA), 1996). The aims of the present study were therefore as follows: (1) to investigate the effects of Cr (exogenous $CrCl_3$ salts) on the growth and Cr accumulation in the edible part of carrot plants grown in a wide range of Chinese soils, and then (2) to derive soil thresholds for carrot cropping based either on food safety standard or on carrot yield reduction.

2. Materials and Methods

2.1. Soil description and experimental design

A total of twenty soils covering a wide range of soil properties were sampled from the surface (0 – 20 cm topsoil) of farmlands throughout China. The soils were air-dried at room temperature, homogenized, and passed through a 2 mm sieve prior to use. Selected physicochemical properties of the soils are shown in Table 1.

A greenhouse pot 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 Cr ($CrCl_3$ solution) on May 26, 2011. The Cr limits for soils used for vegetable production are 150.0, 200.0, and 250.0 mg kg^{-1} for pH < 6.5, pH 6.5 – 7.5, and pH > 7.5, respectively, according to the soil environmental quality standard of China (State Environmental Protection Administration of China, 1995). Therefore, three Cr dosages for each pH-based group of soils were used: the control (CK, no Cr added to soil), low-Cr (Cr1, three-quarters of the soil Cr limit, i.e. 112.5, 150.0, and 187.5 for pH < 6.5, 6.5–7.5, and > 7.5, respectively), and high-Cr (Cr2, equal to the soil Cr limit, i.e. 150.0, 200.0, and 250.0 for pH < 6.5, 6.5 – 7.5, and > 7.5, respectively). The soil was then left for aging under natural conditions for three months. During the aging period, soil water was maintained at 80 percent maximum water holding capacity. These pots were arranged in a randomized complete block design with three replicates.

To ensure normal growth of carrot plants and exclude the possible influence of nutrient deficiency, basal fertilizers were applied and mixed thoroughly with soil after the aging period. 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 New Kuroda, known to be the most widely used for being eaten fresh and processed for export in China (Li and Yu, 2004), were purchased from Nanjing Qiutian Seed Company and sown directly to the soils on August 24, 2011. The number of seedlings was thinned to three per pot after their emergence. The soils were watered to maintain normal growth and the accumulation of excess water at the bottom of the pots was avoided during the carrot growing period.

2.2. Soil and plant analysis

Carrot was harvested at maturity on December 21, 2011. 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 weight (FW) was recorded, and the samples were homogenized using a Retsch-grinder (GM 200, Germany). Subsamples of edible part were digested with HNO_3:H_2O_2 (4:3) in high pressure sealed digestion vessels according to Determination of Chromium in Foods, National Food Safety Standard of China (GB 5009.123-2003). After the aging period, soil samples were collected, air-dried, and ground to pass through a 0.149-mm sieve for total Cr analysis (Lu, 2000). The phytoavailable Cr extracted with 0.005 M DTPA (Lindsay and Norvell, 1978) in soil samples after harvest was also determined.

Soil pH, cation exchange capacity, soil texture, the contents of soil organic carbon, and total Fe, Mn and Al oxides were analyzed according to the routine analytical methods of agricultural chemistry in soil (Lu, 2000). The total Cr concentration in soils 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 94 to 107 percent and 91 to 103 percent, respectively, throughout the analysis procedure. All chemical reagents used in the Cr analysis were of guaranteed reagent (GR) grade, and in the analysis of other soil properties were of analytical reagent (AR) grade.

Table 1
Main physicochemical properties of the soils.

Soil[a]	Location	Coordinates	pH	OC[b] (g kg^{-1})	CEC[c] (cmol kg^{-1})	Clay (< 0.002 mm, %)	Fe_{OX}[d] (g kg^{-1})	Mn_{OX}[e] (g kg^{-1})	Al_{OX}[f] (g kg^{-1})	Background Cr[g] (mg kg^{-1})
1	Guiyang, Guizhou	26°26'N, 106°31′E	4.67	20.6	15.4	55.8	86.2	0.43	195	55.5
2	Nanning, Guangxi	22°36'N, 108°21'E	4.81	14.6	7.63	36.7	54.7	0.10	115	40.4
3	Yingtan, Jiangxi	28°12'N, 116°56'E	4.84	5.43	9.31	45.8	50.2	0.28	131	68.9
4	Chongqing	29°49'N, 106°24'E	4.99	9.92	16.9	20.2	43.8	0.92	138	51.7
5	Shenyang, Liaoning	41°30'N, 123°28'E	5.35	8.81	15.8	22.4	37.9	1.05	132	52.5
6	Daye, Hubei	30°05'N, 114°59'E	5.68	10.1	12.3	29.6	95.7	0.89	170	23.1
7	Nanjing, Jiangsu	32°06'N, 119°00'E	6.28	12.9	12.1	15.9	27.9	0.34	90.8	45.3
8	Qiyang, Hunan	28°16'N, 112°42'E	6.31	16.5	14.0	31.3	46.9	0.88	123	54.7
9	Gongzhuling, Jilin	43°37'N, 124°54'E	6.52	14.0	24.9	33.6	29.8	1.43	123	48.5
10	Haikou, Hainan	19°58'N, 110°15'E	6.83	6.06	4.53	17.8	45.9	0.49	87.0	37.5
11	Tianjin	39°41'N, 117°25'E	6.93	9.90	24.1	36.5	40.2	1.16	139	56.7
12	Lhasa, Xizang	29°40'N, 91°06'E	7.01	12.1	8.53	10.1	36.6	0.95	120	31.9
13	Fuzhou, Fujian	26°12'N, 118°51'E	7.12	9.32	10.2	20.9	39.7	0.38	204	4.60
14	Gongzhuling, Jilin	43°54'N, 124°59'E	7.30	15.2	23.2	33.4	33.1	0.90	129	46.3
15	Shuangliao, Jilin	43°25'N, 123°31'E	7.88	21.6	14.4	6.35	22.2	0.76	94.9	27.9
16	Suzhou, Jiangsu	31°19'N, 120°28'E	8.04	5.55	8.18	13.8	43.8	1.09	117	58.0
17	Shijiazhuang, Hebei	38°23'N, 114°42'E	8.23	8.58	9.16	9.16	32.1	1.04	122	56.2
18	Hohhot, Inner Mongolia	41°19'N, 109°56'E	8.37	9.54	9.70	8.34	28.2	0.94	123	48.1
19	Lanzhou, Gansu	35°52'N, 104°14'E	8.41	7.38	7.63	11.3	42.3	1.11	107	53.5
20	Urumqi, Xinjiang	44°30'N, 87°45'E	8.67	4.30	6.65	10.3	32.2	1.11	115	45.6

[a] Soil numbers were sequenced in the order of increasing pH. The soil sampled from Xi'an, Shaanxi Province (soil 20 in Ding et al. (2013)) was excluded here since it failed to develop carrot bulb under Cr addition treatments.
[b] Organic carbon.
[c] Cation exchange capacity (buffered).
[d] Total Fe oxide.
[e] Total Mn oxide.
[f] Total Al oxide.
[g] Presented as total Cr.