Phytoavailability of Cadmium (Cd) to Pak Choi (Brassica chinensis L.) Grown in Chinese Soils: A Model to Evaluate the Impact of Soil Cd Pollution on Potential Dietary Toxicity

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Abstract
Food chain contamination by soil cadmium (Cd) through vegetable consumption poses a threat to human health. Therefore, an understanding is needed on the relationship between the phytoavailability of Cd in soils and its uptake in edible tissues of vegetables. The purpose of this study was to establish soil Cd thresholds of representative Chinese soils based on dietary toxicity to humans and develop a model to evaluate the phytoavailability of Cd to Pak choi (Brassica chinensis L.) based on soil properties. Mehlich-3 extractable Cd thresholds were more suitable for Stagnic Anthrosols, Calcareous, Ustic Cambosols, Typic Hapludalfs, Udic Ferrisols and Periudic Argosols with values of 0.30, 0.25, 0.18, 0.16, 0.15 and 0.03 mg kg⁻¹, respectively, while total Cd is adequate threshold for Mollisols with a value of 0.86 mg kg⁻¹. A stepwise regression model indicated that Cd phytoavailability to Pak choi was significantly influenced by soil pH, organic matter, total Zn and Cd concentrations in soil. Therefore, since Cd accumulation in Pak choi varied with soil characteristics, they should be considered while assessing the environmental quality of soils to ensure the hygienically safe food production.

Introduction
Cadmium (Cd) is an important environmental pollutant toxic to animals and human beings. It is one of the most mobile elements, among all the toxic heavy metals [1]. Cadmium is not required for plants growth or reproduction, however its bioaccumulation and subsequent accrual in the food chain surpasses all other trace elements due to its high mobility in soil [2]. It is the most toxic element in the environment and even at low concentrations is very toxic to living cells and considered as carcinogenic [3]. In humans, Cd exposure can result in multiple adverse effects, such as testicular damage, renal and hepatic dysfunction, etc. [3]. Moreover, Cd is implicated in the development of cancer, phytotoxic at higher levels of concentrations [4] and classified as a type I carcinogen by the International Agency for Cancer Research [5]. Significant quantities of Cd can be transferred from contaminated soil to plants [6]. Therefore, crops produced from Cd contaminated soils may be unsuitable or even detrimental for animal and human consumption [7].

Vegetables are an important component of human diet since they contain proteins, carbohydrates as well as minerals and vitamins [8]. The proportion of vegetables consumed in the total diet has been increased with the improvement of living standards. However, vegetables are also one of the most important pathways through which heavy metals enter the food chain and affect human health. Leafy vegetables can accumulate higher concentrations of Cd than other crops [9,10]. Leafy vegetables are known to accumulate higher concentrations of Cd in the edible parts even when grown in soils containing low concentrations of Cd [11]. Pak choi (Brassica chinensis L.), also known as Chinese cabbage, is a popular leafy vegetable, grown and consumed worldwide. Therefore, it is imperative to control Cd concentrations in Pak choi, especially in its edible parts to ensure food safety. To limit the transfer of soil Cd into the edible parts of Pak choi, an understanding of its accumulation characteristics is required. Currently, there is an elevated concern over Cd accumulation in food and its potential risks to human health [12]. Cadmium accumulation and distribution varies among vegetable cultivars.
and tissues [13]. However, the accumulation and distribution of Cd in vegetables grown in a diversity of soil types were rarely studied [14].

About one fifth of agricultural land is contaminated by Cd, lead (Pb) and arsenic (As) in China [15]. Moreover, it was reported that about 20% of farm lands in China are contaminated with heavy metals and Cd contamination accounts for more than 1.3×10^7 ha of the total affected area [16,17]. Cadmium uptake by rice (Oryza sativa L.) and vegetables from soil is the initial source of exposure for human beings [18,19]. Therefore, there are environmental concerns of soils, food safety and human health for the present and future agricultural and environmental sustainability of world vegetable supplies. As, only a small fraction of total trace metals in soil is available for plant absorption, it is widely accepted that the total metal content in soils is neither a viable indicator of phytoavailability nor an adequate tool to assess the potential risk of dietary toxicity [12]. Tracy and Sheila [20] reported that extractable Cd content in soil may be an improved indicator of bioavailability and toxicity than the total contents and toxicity and availability of metals differed among soils types. Metal uptake and translocation studies were conducted for different crops under varying soil conditions, to further understanding uptake and the transport mechanisms [21,22].

To ensure the food safety and environmental quality of soils, guidelines for permissible concentrations of Cd in agricultural soils need to be established. Due to limited number of studies, the soil environmental quality guidelines for heavy metals in farmland soils developed and applied in the world are still based on total metal contents of soil. Minimal attention has been focused on metal translocation from soils to plants, which are used as food crops, and absorption of metals in food plants to concentration that does not cause phytotoxicity symptoms [23]. This study was conducted in seven Chinese soil types to establish direct relationship of Cd level in such contaminated soils and Cd uptake in Pak choi. The main objectives were to establish soil Cd thresholds for representative Chinese soils based on human dietary toxicity and to determine the relationships between several soil properties and Cd accumulation in Pak choi. This information will be useful in establishing soil protection guidelines to produce hygienically safe vegetables.

Materials and Methods

Ethics Statement

The soils used in this study were agricultural soils. No specific permissions were required for the described locations. We confirm that the field studies did not involve endangered or protected species.

Soil Collection and Analysis

Seven Chinese soils were selected for this study. Udic Ferrisols, Mollisols, Periudic Argosols, Typic Haplustalfs, Ustic Cambosols, Calcaric Regosols and Stagnic Anthrosols were collected from Chinese cities of Guilin (104°40′–119°45′E, 2,418′– 25°41′N), Harbin (125°42′– 130°10′E, 44°04′– 46°40′N), Huzhou (119°14′– 120°29′E, 30°22′– 31°11′N), Zhanjiang (110°08′–110°77′E, 20°33′–21°62′N), Qidu (116°51′–117°13′E, 33°29′–35°49′N), Ya’an (102°37′–103°12′E, 29°23′–30°37′N) and Jiaxing (120°7′–121°02′E, 30°5′–30°77′N), respectively. Soils samples were taken at a depth of up to 20 cm from the upper horizon. Each sample was air-dried, ground, and screened through two mm sieve before laboratory analysis. Soil pH, cation exchange capacity, organic matter contents, and particle size density were measured by using previously described methods [24–27]. Physicochemical properties of these soils are reported (Table 1).

Cadmium Spiking and Aging

Soil samples of Mollisols, Periudic Argosols, Stagnic Anthrosols and Ustic Cambosols were spiked with Cd as Cd(NO₃)₂ in an aqueous solution at loading rates of 1.0, 2.0, 4.0, 6.0 and 8.0 mg Cd kg⁻¹ soil along with an untreated control (Ck), the background values of Cd concentration was below 0.50 mg kg⁻¹ in these soil. However, the Udic Ferrisols, Typic Haplustalfs and Calcaric Regosols soil samples, with the background values of Cd concentration above 0.50 mg kg⁻¹, were spiked with Cd to establish the contamination levels of 2.0, 4.0, 6.0 and 8.0 mg Cd kg⁻¹ soil along with the untreated control (Ck). Soil moisture was maintained up to 70% of its water-holding capacity by using distilled water. All the spiked soils were aged for one year subsequent to greenhouse experimentation. After one year aging period, the concentrations of total Cd, and Mehlich-3 extractable Cd were determined in each of the spiked soils.

Containerized Experiment

A containerized experiment was performed in greenhouse by growing Pak choi (Brassica chinensis L.) during March – April, 2012 at Zhejiang University, Hangzhou, China. Seed of Pak choi was obtained from the Zhejiang Seed Co. Hangzhou, China. Seeds were washed with distilled water and air-dried prior to sowing. Seeds were germinated in dark at 25°C and transplanted into quartz sand bed to establish seedlings. Four healthy, uniform and 21-day-old seedlings were transplanted into plastic containers with a diameter of 18 cm and height of 17 cm. Each container had 3 kg of soil. Fertilizers were applied at the rates of 0.4 g of N as CO(NH₂)₂ and 0.2 g P as KH₂PO₄ per kg of soil. The experiment was carried out in a completely randomized design (CRD). Treatments were established in triplicate, and the containers were randomly arranged in a greenhouse bench under controlled conditions of 16 h of light at 30°C and 8 h of dark at 22°C. Plants were monitored daily and watered as necessary.

Plant Sample Collection

Pak choi was harvested after 30 days from transplanting. The plants of Pak choi were removed from each container and separated into root and shoots (including stems and leaves). Roots and shoots of Pak choi were first washed with tap water and then with ultrapure distilled water, to remove all visible soil particles. Clean plant samples were first blotted dry, and then dried at 70°C for 72 h in an oven. Dry shoot weight of samples was recorded. Dry plant samples were ground to pass through a 60 mm sieve using an agate mill prior to Cd concentration analysis.

Total Cd of Soil and Plant

For determination of total Cd concentration in soil, 0.20 g of soil samples was digested with HNO₃–HF–HClO₄ (5:1:1) [4]. Plant samples, 0.20 g of shoots for each treatment was digested with HNO₃–H₂O₂ (5:1). After cooling the digest was transferred to a volumetric flask, diluted with distilled water to 50 mL [28]. The concentrations of Cd in the filtrate were determined using inductively coupled plasma–mass spectrometry (ICP-MS, Agilent, 7500a, CA, USA). The ICP-MS was operated at the following
## Table 1. Basic Chemical and Physical Characteristics of Seven Chinese Soils.

| Soil Types               | pH    | OM (g kg\(^{-1}\)) | CEC (cmol kg\(^{-1}\)) | Total Cd (mg kg\(^{-1}\)) | Total Zn (mg kg\(^{-1}\)) | Silt (%) | Clay (%) |
|--------------------------|-------|--------------------|-------------------------|---------------------------|----------------------------|----------|----------|
| Mollisols                | 6.49  | 4.10               | 0.51                    | 0.01                      | 1.44                       | 60.2     | 19.2     |
| Ustic Cambosols          | 7.45  | 7.54               | 0.02                    | 0.59                      | 2.16                       | 65.2     | 4.6      |
| Eutic Cambosols          | 5.16  | 2.14               | 0.06                    | 0.47                      | 2.04                       | 73.0     | 19.2     |
| Stagnic Anthrosols       | 6.49  | 4.10               | 0.51                    | 0.01                      | 1.44                       | 60.2     | 19.2     |
| Periodic Argosols        | 7.45  | 7.54               | 0.02                    | 0.59                      | 2.16                       | 65.2     | 4.6      |
| Udic Ferrisols           | 5.16  | 2.14               | 0.06                    | 0.47                      | 2.04                       | 73.0     | 19.2     |
| Calcaric Regosols        | 6.49  | 4.10               | 0.51                    | 0.01                      | 1.44                       | 60.2     | 19.2     |

### Mehlich-3 Extractable Cd in Soils

Mehlich-3 extractable Cd in soils was determined following the extraction method described by Mehlich [29]. Briefly, 5 g (0.2 mm sieved) of dry soil was shaken with 50 mL of Mehlich-3 solution (0.2 mol L\(^{-1}\) CH\(_3\)COOH, 0.25 mol L\(^{-1}\) NH\(_4\)NO\(_3\), 0.015 mol L\(^{-1}\) NH\(_4\)F, 0.013 mol L\(^{-1}\) HNO\(_3\), 0.001 mol L\(^{-1}\) EDTA) for 5 min (200 rpm) at 25°C. The suspension was centrifuged at 5000 rpm for 10 min and filtered through 0.45 μm filter paper. The same procedure without samples was used as control and three replications were conducted for each soil sample. The Cd concentration in the filtrate was analyzed by inductively coupled plasma–mass spectrometry (ICP-MS, Agilent 7500a, CA, USA).

### Quality Control for Cd Analysis

Quality assurance and quality control (QA/QC) for Cd in soil and Pak choi were conducted by determining Cd contents in the certified reference materials (soil GSBZ 50013-88 and plant GBW-07402) respectively, approved by General Administration of Quality Supervision, Inspection and Quarantine of the People’s Republic of China (AQSIQ) and National Center for Reference Materials. The analytical results showed a recovery rate of 97.3% and 102.1% respectively.

### Derivation of Soil Cd Thresholds for Potential Dietary Toxicity in Pak choi

For ensuring the environmental and food safety for human beings, an effort has been made to develop guidelines for acceptable concentrations of potentially harmful Cd in seven agricultural soils types of China. In this context, the amounts of Cd in Pak choi above than threshold level of food safety are adversely affecting humans are critical. Since, Cd bioavailability differed among soil types, the focus was on the development of soil Cd thresholds for representative Chinese soils based on food safety, Provisional Tolerable Weekly Intake (PTWI) of Cd recommended by FAO/WHO Joint Expert Committee on Food Additives, is 7 μg kg\(^{-1}\) of body weight [30]. Estimated daily intake of metal (EDIM) was determined by the following equation.

\[
EDIM = \frac{C_{Cadmium} \times C_{factor} \times D_{daily intake}}{B_{average weight}}
\]

Where, \(C_{Cadmium}\), \(C_{factor}\), \(D_{daily intake}\) and \(B_{average weight}\) represent average Cd concentration in Pak choi (μg kg\(^{-1}\)), conversion factor, daily consumption of Pak choi (g) and average body weight (kg) of the adult consumers, respectively. Average daily consumption of Pak choi for adults was considered to be 0.345 kg person\(^{-1}\) d\(^{-1}\) [31] and a conversion factor 0.085 was used to convert fresh Pak choi weight to dry weight [32]. Average body weight of adult was considered to be 60 kg as motioned in previous reports [30]. According to the above equation of EDIM, the provisional tolerable daily intake of Cd for Pak choi was 2.04 mg kg\(^{-1}\) on a dry weight basis. Soi Cd threshold levels for potential dietary toxicity from Pak choi were calculated according to the tolerable daily dietary intake level of Cd (2.04 mg kg\(^{-1}\)) and the regression equations.
Statistical Analysis

Stepwise multiple regression analysis, single linear regression and one-way analysis of variance (ANOVA) were performed using the statistical software package SPSS (version 18.0). All values reported in this work are means of three independent replications. Treatment means were separated by least significant difference (LSD) test, at 5% level.

Results

Characteristics of Soils

Soils evaluated were representative of most of Chinese soil types, pH range of soils were strongly acidic to mild alkaline. Chemical and physical characteristics varied among the seven soils. Highest total Cd and Zn concentrations (background value) were observed in Udic Ferrisols and Stagnic Anthrosols respectively. Mollisols contained the highest amount of organic matter and exhibited an elevated cation exchange capacity as well (Table 1).

Mehlich-3 Extractable Cd in Soils after Aging of 1 Year

Mehlich-3 extractable Cd content increased significantly with increasing Cd spiking levels in all the seven soils. Mehlich-3 extractable Cd ranged from 0.16–3.95 mg kg\(^{-1}\) in these soils under different Cd levels (Table 2). The Cd contents varied significantly among these soils, decreasing in order: Periodic Argosols > Typic Haplustalfs > Udic Ferrisols > Stagnic Anthrosols > Mollisols > Ustic Cambosols > Calcari Regosols. Mehlich-3 extractable Cd concentration was greater at higher rates of Cd spiking in each soil. These results indicated that minimum and maximum extractability of Cd was found in Calcaric Regosols and Periudic Argosols respectively under different Cd levels (Table 2). The Cd contents varied significantly among soils at different Cd levels and soil types. Roots exhibited the higher Cd contents as compared with shoots biomass of Pak choi grown in Ustic Cambosols, Udic Ferrisols, Stagnic Anthrosols and Calcari Regosols soils, indicating low phytoavailability of Cd in these soils (Table 3). The stimulating effect of Cd on shoot biomass of Pak choi occurred at 1 mg kg\(^{-1}\) and 2 mg kg\(^{-1}\) in Ustic Cambosols and Mollisols respectively, whereas in Stagnic Anthrosols, it occurred at 4 mg kg\(^{-1}\). The dry weight of Pak choi shoots at 8 mg kg\(^{-1}\) Cd generally decreased in order of: Calcari Regosols > Mollisols, Stagnic Anthrosols > Ustic Cambosols > Udic Ferrisols > Periodic Argosols > Typic Haplustalfs (Table 3).

Accumulation and Distribution of Cadmium in Pak choi

Cadmium concentration in the shoots and roots of Pak choi varied significantly among soils at different Cd levels and soil types. Roots exhibited the higher Cd contents as compared with shoots biomass of Pak choi grown in Ustic Cambosols, Udic Ferrisols, Stagnic Anthrosols and Calcari Regosols soils, indicating low phytoavailability of Cd in these soils (Table 3). The stimulating effect of Cd on shoot biomass of Pak choi occurred at 1 mg kg\(^{-1}\) and 2 mg kg\(^{-1}\) in Ustic Cambosols and Mollisols respectively, whereas in Stagnic Anthrosols, it occurred at 4 mg kg\(^{-1}\). The dry weight of Pak choi shoots at 8 mg kg\(^{-1}\) Cd generally decreased in order of: Calcari Regosols > Mollisols, Stagnic Anthrosols > Ustic Cambosols > Udic Ferrisols > Periodic Argosols > Typic Haplustalfs (Table 3).

Biomass Yield of Pak choi

Generally, Pak choi had tolerance to Cd toxicity in Mollisols, Stagnic Anthrosols and Calcari Regosols soils, indicating low phytoavailability of Cd in these soils. Shoot biomass of Pak choi under different Cd treatments of these soils did not decrease significantly as compared with their respective controls. However, the shoot biomass of Pak choi grown in Ustic Cambosols, Udic Ferrisols, Periodic Argosols and Typic Haplustalfs decreased significantly as compared with the control indicating higher phytoavailability of Cd in these soils (Table 3). The Cd contents varied significantly among soils at different Cd levels and soil types. Roots exhibited the higher Cd contents as compared with shoots biomass of Pak choi grown in Ustic Cambosols, Udic Ferrisols, Stagnic Anthrosols and Calcari Regosols soils, indicating low phytoavailability of Cd in these soils (Table 3). The stimulating effect of Cd on shoot biomass of Pak choi occurred at 1 mg kg\(^{-1}\) and 2 mg kg\(^{-1}\) in Ustic Cambosols and Mollisols respectively, whereas in Stagnic Anthrosols, it occurred at 4 mg kg\(^{-1}\). The dry weight of Pak choi shoots at 8 mg kg\(^{-1}\) Cd generally decreased in order of: Calcari Regosols > Mollisols, Stagnic Anthrosols > Ustic Cambosols > Udic Ferrisols > Periodic Argosols > Typic Haplustalfs (Table 3).

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Table 2. Mehlich-3 Extractable Cd Contents (mg kg\(^{-1}\)) in Seven Chinese Soils at the onset of Containerized Experiment after Aging of 1 year.

| Cd conc. (mg kg\(^{-1}\)) | Mollisols | Ustic Cambosols | Stagnic Anthrosols | Periodic Argosols | Typic Haplustalfs |
|--------------------------|-----------|-----------------|-------------------|------------------|-----------------|
| 1                        | 0.46±0.06c | 0.15±0.06c      | 0.22±0.06c        | 0.16±0.06c       | 0.31±0.12d      |
| 2                        | 0.19±0.06c | 0.19±0.06c      | 0.21±0.06c        | 0.19±0.06c       | 0.31±0.12d      |
| 4                        | 0.62±0.06c | 0.62±0.06c      | 0.62±0.06c        | 0.62±0.06c       | 0.31±0.12d      |
| 6                        | 2.21±0.09a | 2.21±0.09a      | 2.21±0.09a        | 2.21±0.09a       | 0.31±0.12d      |
| 8                        | 2.89±0.14a | 2.89±0.14a      | 2.89±0.14a        | 2.89±0.14a       | 0.31±0.12d      |

Mean values followed by different letters within the same column are significantly different at P < 0.05.

Table 3. Accumulation of Cd (mg kg\(^{-1}\)) by Pak choi after Aging of 1 year.

| Cd conc. (mg kg\(^{-1}\)) | Mollisols | Ustic Cambosols | Stagnic Anthrosols | Periodic Argosols | Typic Haplustalfs |
|--------------------------|-----------|-----------------|-------------------|------------------|-----------------|
| 1                        | 0.19±0.06c | 0.19±0.06c      | 0.19±0.06c        | 0.19±0.06c       | 0.19±0.06c      |
| 2                        | 0.21±0.06c | 0.21±0.06c      | 0.21±0.06c        | 0.21±0.06c       | 0.21±0.06c      |
| 4                        | 0.62±0.06c | 0.62±0.06c      | 0.62±0.06c        | 0.62±0.06c       | 0.62±0.06c      |
| 6                        | 2.21±0.09a | 2.21±0.09a      | 2.21±0.09a        | 2.21±0.09a       | 2.21±0.09a      |
| 8                        | 2.89±0.14a | 2.89±0.14a      | 2.89±0.14a        | 2.89±0.14a       | 2.89±0.14a      |
bioavailability. The lowest and highest Cd concentrations in the Pak choi tissues were at the highest (8 mg kg\(^{-1}\)) level of Cd in Calcaric Regosols and Periodic Argosols, respectively. Cadmium concentrations in Pak choi followed an order of: Periodic Argosols > Typic Haplustalfs > Udic Ferrisols > Ustic Cambosols > Mollisols > Stagnic Anthrosols > Calcaric Regosols at 8 mg kg\(^{-1}\) soil Cd level (Table 4).

**Relationship between Mehlich-3 Extractable Cd in Soils and Pak choi Cd Content**

Cadmium concentrations in shoots of Pak choi were significantly correlated to total Cd and Mehlich-3 extractable Cd contents in soils \(R^2 = 0.95\) to 0.99, and 0.97 to 0.99 respectively). Cadmium concentrations in Pak choi shoots were best related to total Cd content in Mollisols \(R^2 = 0.99\). Whereas, the Cd concentrations of Pak choi shoots were best correlated to Mehlich-3 extractable Cd in Ustic Cambosols, Stagnic Anthrosols, Periudic Argosols, Udic Ferrisols, Typic Haplustalfs and Calcaric Regosols with \(R^2 = 0.97, 0.99, 0.99, 0.99, 0.99, 0.99\) and 0.96, respectively (Table 5).

**Soil Cd Thresholds for Potential Dietary Toxicity in Pak choi**

Total soil Cd thresholds for potential dietary toxicity from the consumption of Pak choi conform to an order of: Calcaric Regosols > Stagnic Anthrosols > Ustic Cambosols > Mollisols > Udic Ferrisols > Typic Haplustalfs > Periudic Argosols, and were 1.25, 1.16, 1.02, 0.96, 0.72, 0.70 and 0.12 mg kg\(^{-1}\), respectively. Mehlich-3 extractable Cd thresholds were 0.30, 0.25, 0.23, 0.18, 0.16, 0.15 and 0.03 mg kg\(^{-1}\) and decreased in the following order of: Stagnic Anthrosols > Calcareous > Mollisols > Ustic Cambosols > Typic Haplustalfs > Udic Ferrisols > Periodic Argosols, respectively (Table 6).

**Discussion**

**Biomass Yield of Pak choi**

Dry weight of Pak choi did not decrease significantly under different Cd levels (Ck to 8.0 mg kg\(^{-1}\)) in Mollisols, Stagnic Anthrosols and Calcaric Regosols and even increased at 1, 2 and 4.0 mg kg\(^{-1}\) of treatment levels. Similar stimulatory responses of biomass to Cd exposure have also been reported in several plant species [33,34]. The stimulatory effect of Cd on plant biomass may be explained by various mechanisms, for examples, metal ions can serve as enzyme activators in cytokinins metabolism, which stimulates the growth of plants, [35] and a low dose of metal exposure may cause changes in cytokinins and plant hormones that regulate growth and development of plants [36]. Kaminek [36] reported that cytokinins may delay senescence by maintaining chlorophyll production and photosynthetic activity in plant leaves.

Cd exposure may cause changes to various physiological and biochemical processes in plant tissues, such as, reduction in dry biomass may be due to the negative effects of Cd on the roots, and plants could not take up nutrients to continue their normal activities. It has been well reported that Cd can reduce plant growth and development by interfering in various metabolic processes, such as, inhibition of the proton pump, reduction in root elongation, and damage to photosynthetic activity [37,38]. The excess amount of Cd in soil may be responsible for causing disturbances in mineral nutrition and carbohydrate metabolism [39]. Shoot biomass of Pak choi grown in Ustic Cambosols, Udic Ferrisols, Periudic Argosols and Typic Haplustalfs decreased significantly as compared with the control. The inhibitory effect of

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**Table 3. Dry Biomass (g plant\(^{-1}\)) of Pak choi Shoots Grown on Seven Chinese Soils with Different Loading Rates of Cd.**

| Soil Type          | Ck   | 1.25 | 2.5 | 4.0  | 8.0  |
|--------------------|------|------|-----|------|------|
| Mollisols          | 1.25 | 1.91 | 1.66 | 1.60 | 1.55 |
| Ustic Cambosols    | 1.34 | 1.47 | 1.47 | 1.38 | 1.03 |
| Stagnic Anthrosols | 1.45 | 1.53 | 1.53 | 1.28 | 1.03 |
| Typic Haplustalfs  | 1.54 | 1.66 | 1.66 | 1.60 | 1.55 |
| Periudic Argosols  | 1.25 | 1.33 | 1.33 | 1.38 | 1.04 |
| Udic Ferrisols     | 1.45 | 1.53 | 1.53 | 1.28 | 1.03 |
| Calcaric Regosols  | 1.54 | 1.66 | 1.66 | 1.60 | 1.55 |

Mean values followed by different letters within the same column are significantly different at P < 0.05.
Cadmium (Cd) is a heavy metal that can be toxic to human health, particularly when present in soil and can be taken up by plants. It is also a limiting factor to crop production and a potential source of dietary exposure. Understanding the distribution and bioavailability of Cd in soil is crucial for agriculture and public health. In this study, the authors investigated the phytoavailability of Cd to Pak choi, a commonly consumed vegetable in China, grown in soils with different pH levels. They compared Mehlich-3 extraction technique with the more costly EPA method to assess the predictive capabilities of both methods.

The study showed a high correlation coefficient ($R^2 > 0.97$) between Mehlich-3 Cr and Cr $L$, which is a strong acid digestion method. Moreover, it was evidenced by high correlation coefficients ($R^2 > 0.97$) between Mehlich-3 Cr and Cr $L$, which is in agreement with our previous studies, which reflected a high linear correlation ($R^2 > 0.97$) between Mehlich-3 Cr and Cr $L$.

### Accumulation and Distribution of Cadmium in Pak choi

Variations of Cd accumulation in Pak choi grown in different soils with different pH may be due to the difference in bioavailability of Cd in each soil. Lai and Chen [41] reported that Cd concentration in Pak choi shoots was up to 83 mg kg$^{-1}$ DW with an application of soil Cd to up to 20 mg kg$^{-1}$. Moreover, it was observed that accumulation of the Cd in rice shoot ranged from 67.9 to 241.7 mg kg$^{-1}$.

### Relationship between Mehlich-3 Extractable Cd in Soils and Pak choi Cd Content

Mehlich-3 extraction technique appeared efficient to assess Cd phytoavailability to Pak choi, grown in seven textured soils, as evidenced by high correlation coefficients ($R^2 > 0.97$). This is in agreement with our previous studies, [43,28] which reflected a high linear correlation ($R^2 > 0.97$) between Mehlich-3 Cr and Cr $L$. Moreover, it was evidenced by high correlation coefficients ($R^2 > 0.97$) between Mehlich-3 Cr and Cr $L$, which is in agreement with our previous studies, which reflected a high linear correlation ($R^2 > 0.97$) between Mehlich-3 Cr and Cr $L$.

### Soil Cd Thresholds for Potential Dietary Toxicity in Pak choi

Cadmium concentrations in the shoots of Pak choi were significantly correlated to total Cd and Mehlich-3 extractable Cd contents in soils, with $R^2$ values of 0.95 to 0.99, and 0.97 to 0.99, respectively. From this investigation, Cd contents in Pak choi shoots were correlated to total Cd content in Mollisols ($R^2$ values of 0.99). Cadmium concentrations of Pak choi shoot contents were highly correlated to Mehlich-3 extractable Cd in Ustic Cambosols, Stagnic Anthrosols, Periudic Argosols, Udric Ferrisols, Typic Hapludalfs, Calcaric Regosols, Haplustalfs, and Calcaric Regosols with $R^2$ values of 0.95 to 0.99.

### Summary

Cadmium is a heavy metal that can be toxic to human health if present in soil and can be taken up by plants. Understanding the distribution and bioavailability of Cd in soil is crucial for agriculture and public health. In this study, the authors investigated the phytoavailability of Cd to Pak choi, a commonly consumed vegetable in China, grown in soils with different pH levels. They compared Mehlich-3 extraction technique with the more costly EPA method to assess the predictive capabilities of both methods. The Mehlich-3 extraction technique appeared efficient to assess Cd phytoavailability to Pak choi, grown in seven textured soils, as evidenced by high correlation coefficients ($R^2 > 0.97$). This is in agreement with our previous studies, [43,28] which reflected a high linear correlation ($R^2 > 0.97$) between Mehlich-3 Cr and Cr $L$. Furthermore, it was evidenced by high correlation coefficients ($R^2 > 0.97$) between Mehlich-3 Cr and Cr $L$, which is in agreement with our previous studies, which reflected a high linear correlation ($R^2 > 0.97$) between Mehlich-3 Cr and Cr $L$. The Mehlich-3 extraction technique was positively correlated with the more costly EPA test, and it could be developed as a less expensive and easily conductable technique [46].
Typic Haplustalfs, Udic Ferrisols, Periudic Argisols and were 1.25, 1.16, 1.02, 0.86, 0.72, 0.70 and 0.12 mg kg\(^{-1}\), respectively. Mehlich-3 extractable Cd thresholds decreased in the following order of: Stagnic Anthrosols, Calcareous Mollisols, Ustic Cambosols, Udic Ferrisols, Typic Haplustalfs, Periudic Argosols and were 0.30, 0.25, 0.23, 0.18, 0.16, 0.15 and 0.03 mg kg\(^{-1}\), respectively (Table 6).

Cadmium concentrations in Pak choi shoots, were highly correlated to total Cd content in Mollisols with the threshold levels of 0.86 mg kg\(^{-1}\) with a \(R^2 = 0.99\). However, the Cd concentrations of Pak choi shoots were best related to Mehlich-3 extractable Cd in Stagnic Anthrosols, Calcareous Mollisols, Ustic Cambosols, Udic Ferrisols, Typic Haplustalfs and Periudic Argosols with thresholds values of 0.30, 0.25, 0.23, 0.18, 0.16, 0.15 and 0.03 mg kg\(^{-1}\), \(R^2\) values of 0.97, 0.99, 0.99, 0.98, 0.98 and 0.98, respectively. Based on the wide range of applicability and the simplicity of extraction method, it is proposed that Mehlich-3 extractable Cd is more suitable to be used as soil Cd thresholds for potential dietary toxicity in Pak choi. Our previous study evaluated the phytoavailability of Cd as compared to other crops [9,10]. Therefore the predicted threshold is even lower than background value of Cd in soil; it means that there is a risk for dietary toxicity from Pak choi grown on it, even with the background value of total Cd in soil. This kind of information has been reported in our previous study. The threshold of total soil Cd for rice was 0.21 mg kg\(^{-1}\) which was also lower than background value of total Cd in soil [47].

Cadmium levels (Ck, 1, 2, 4, 6, 8 mg kg\(^{-1}\)) used in this investigation represented uncontaminated, lightly contaminated, and moderately Cd polluted soils. Therefore, these levels of Cd contamination are realistic, comparable to those applied in other soil safety risk assessment studies, and thus, the results are applicable in field conditions as well.

**Table 5. Regression Correlation between Cd Contents in the Edible Shoots of Pak choi and Different Forms of Cd in Various Soils.**

| Soil type          | Form of Soil Cd          | Regression equation            | \(R^2\) |
|--------------------|--------------------------|--------------------------------|---------|
| Mollisols          | Total Cd                 | \(y = 2.1706x + 0.1669\)       | 0.99    |
|                    | Mehlich-3 extractable Cd | \(y = 5.7207x + 0.7037\)       | 0.98    |
| Ustic Cambosols    | Total Cd                 | \(y = 2.9358x - 0.9586\)       | 0.96    |
|                    | Mehlich-3 extractable Cd | \(y = 7.0409x + 0.7648\)       | 0.97    |
| Stagnic Anthrosols | Total Cd                 | \(y = 2.2344x - 0.5651\)       | 0.98    |
|                    | Mehlich-3 extractable Cd | \(y = 5.3848x + 0.4458\)       | 0.99    |
| Periudic Argosols  | Total Cd                 | \(y = 11.061x + 0.6961\)       | 0.98    |
|                    | Mehlich-3 extractable Cd | \(y = 22.326x + 1.3968\)       | 0.99    |
| Typic Haplustalfs  | Total Cd                 | \(y = 8.7318x - 4.0825\)       | 0.97    |
|                    | Mehlich-3 extractable Cd | \(y = 19.123x - 8.046\)        | 0.98    |
| Udic Ferrisols     | Total Cd                 | \(y = 6.6697x - 2.7793\)       | 0.97    |
|                    | Mehlich-3 extractable Cd | \(y = 14.873x - 3.773\)        | 0.99    |
| Calcaric Regosols  | Total Cd                 | \(y = 0.8936x + 0.924\)        | 0.95    |
|                    | Mehlich-3 extractable Cd | \(y = 3.5937x + 1.1512\)       | 0.98    |

**Table 6. Soil Cd Threshold Levels for Potential Dietary Toxicity in Edible Part of Pak choi Calculated from the Permissible Limit of Cd (2.04 mg kg\(^{-1}\) DW) in Leafy Vegetables and Regression Equations.**

| Soil Type          | Total Cd (mg kg\(^{-1}\)) | Mehlich-3 extractable Cd (mg kg\(^{-1}\)) |
|--------------------|---------------------------|------------------------------------------|
| Mollisols          | 0.86                      | 0.23                                     |
| Ustic Cambosols    | 1.02                      | 0.18                                     |
| Stagnic Anthrosols | 1.16                      | 0.30                                     |
| Periudic Argosols  | 0.12                      | 0.03                                     |
| Udic Ferrisols     | 0.72                      | 0.16                                     |
| Typic Haplustalfs  | 0.70                      | 0.15                                     |
| Calcaric Regosols  | 1.25                      | 0.25                                     |
Stepwise Regression Model for Predicting Cd Phytoavailability to Pak choi

Many physicochemical properties of soils can influence the heavy metal accumulation in vegetables. For example, the amount of heavy metal uptake from soils was influenced by soil pH, organic matter (OM) content, cation exchange capacity (CEC) and soil texture [49]. The combinations of basic soil properties may explain Cd uptake by plants [50]. By considering this aspect, soil pH, OM, CEC, total soil Cd, total Zn and clay contents were integrated to simulate the combined effects of soil environment on Cd phytoavailability to Pak choi. Stepwise linear regression was conducted and four independent variables pH, total Zn, OM and total Cd significantly influenced the accumulation of Cd in Pak choi plants (Table 7). Both the multiple correlation and partial regression coefficients reached the statistically significant levels at least the 0.05. For multiple linear regression analyses, $R^2$ values could be used to explain variation of the dependents [12]. It was found that $R^2$ value was above 0.97, which means that more than 97% of variation in Cd concentration in Pak choi shoots could be attributed to soil pH, total Zn, OM and total Cd contents in soils (Table 7).

The influence of each factor on Cd concentration of Pak choi (Y) shoots could be further explained by the values of each coefficient [12]. Stepwise regression model revealed that Cd concentration in the Pak choi was enhanced by lower soil pH (negative coefficients showed negative effect and vice versa), total Zn, OM contents and higher total soil Cd. Lower soil pH, zinc, OM and higher soil total Cd are among the factors which enhance the bioavailability Cd contents in soils. Therefore, these three variables had the contradictory effect on Cd phytoavailability to Pak choi. Wang et al. [12] reported that soil characteristics (e.g. pH, CEC and OM) affected the phytoavailability of different heavy metals in soils, and such influences could be considered in the assessment of phytoavailability of heavy metals. There are four parameters involved in this model and then interactions between them were obvious (e.g. Cd concentration in the extractable fraction was correlated with lower soil pH, soil zinc and OM content). Furthermore, the coefficients obtained in the present model can regulate these cross effects and result in an improved model fitting. For example, there was a negative correlation between the soil pH, Zn and OM, these factors had an inverse effect on Cd phytoavailability and soil Cd was the leading factor influencing Cd phytoavailability to Pak choi (coefficient of soil Cd was greater than those of pH, Zn and OM). Our results about soil Cd and pH are in accordance with our recent study which developed an empirical model to correlate the Cd phytoavailability to rice with several soil properties. Soil pH and bioavailable soil Cd were major influencing factors which (pH negatively and soil Cd positively) correlate with the Cd phytoavailability, however total Zn and OM were not included in our previously developed model [47]. Eriksson and Soderstrom [51] reported that the Cd concentration of wheat grain grown on non-calcareous soils of Sweden was positively correlated to soil total Cd and negatively to extractable Zn. A study was conducted on Cd contaminated soils in Taiwan, whereas regression equation was developed to predict Cd concentrations in rice roots by available fractions of Cd and Zn in soil [52]. The negative coefficient of Zn indicated that soil Zn suppressed the uptake of Cd by rice roots in all varieties as Zn has an antagonistic effect on Cd uptake by root [53]. Oliver et al. [54] also observed a significant decrease of Cd up to 50% in wheat grain when 2.5–5.0 kg Zn ha$^{-1}$ was applied to Cd contaminated Australian soils.

Organic matter content was negatively correlated with the accumulation of Cd in Pak choi shoots (Table 7). Organic matter plays an important role in determining the bioavailability and mobility of heavy metals in soils. Organic matter is involved in supplying organic chemicals to the soil solution, which may act as chelates and increase metal bioavailability to plants [55]. However, OM could reduce the bioavailability of heavy metals in soils by adsorption or forming stable complexes with humic substances [56]. Halim et al. [57] reported that addition of humic acid demonstrated a decrease in extractable heavy metal fraction in metal contaminated soils. This could partially explain the negative correlation of organic matter contents and Cd uptake observed in our present study.

Conclusions

The present study concludes that Cd concentration in Pak choi tissues was dependent on soil type. To establish the soil Cd thresholds of potential dietary toxicity from Pak choi, both Cd bioavailability in garden soils and Pak choi tissues should be taken into consideration. The selection of proper soil types for vegetable production can help us to avoid the toxicity of Cd in our daily diet. Stepwise regression model demonstrated that soil pH, organic matter, total Cd and Zinc contents may be the major factors having influence on the phytoavailability of Cd in different textured soils.

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Author Contributions

Conceived and designed the experiments: MTR XY TL. Performed the experiments: MTR RA WX. Analyzed the data: RA AS MA. Contributed reagents/materials/analysis tools: XY. Wrote the paper: MTR RA PJ S.
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