Research Article

Received: 21 September 2020 Revised: 16 February 2021 Accepted article published: 11 March 2021 Published online in Wiley Online Library: 27 March 2021

(wileyonlinelibrary.com) DOI 10.1002/jsfa.11209

A risk-based approach for the safety analysis of eight trace elements in Chinese flowering cabbage (*Brassica parachinensis* L.) in China

Furong Li,[a,b,c] Xu Wang,[a,b] Fuhua Wang,[a,b] Dian Wen,[a,b] Zhichao Wu,[a,b] Yingqiong Du,[a,b] Ruiying Du,[a,b] Brett H Robinson[c*] and Peihua Zhao[a,b]

Abstract

BACKGROUND: Most countries set regulatory values for the total trace element (TE) concentrations in soil, although there is growing interest in using a risk-based approach to evaluate the bioavailable TE using dilute salt extractants or other soil parameters, including pH and organic carbon. The present study compares the current regulatory system (based on total TEs and pH) and a risk-based approach using 0.01 mol L^{-1} $CaCl_2$ to estimate the bioavailable fraction.

RESULTS: In total, 150 paired samples of Chinese flowering cabbages (*Brassica parachinensis*) and their growth soils were collected, and the total and extractable concentrations of chromium (Cr), cadmium (Cd), lead (Pb), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As) and mercury (Hg), as well as soil pH and organic matter content, were measured. No more than 3.33% of the edible parts exceeded Chinese food safety standards, even when growing in soils exceeding the current regulatory thresholds by over 50%. The total soil Cd (1.5 mg kg^{-1}), as well as the extractable concentrations of Cd (0.1 mg kg^{-1}), Ni (0.03 mg kg^{-1}) and Zn (0.1 mg kg^{-1}), are the key factors affecting the TE concentrations in *B. parachinensis*.

CONCLUSION: Our findings suggest that the current soil regulatory guidelines for safe production of *B. parachinensis* are overly strict and conservative. A risk-based approach based on the extractable TE concentrations would provide a better indication for plant uptake of soil TEs and avoid the waste of farmlands that can still produce safe vegetables. Future research should focus on providing crop-specific available TE concentration guidelines to promote effective utilization of farmlands.

Supporting information may be found in the online version of this article.

Keywords: heavy metal; bioavailable; extraction; safe production; micronutrient; security threshold

INTRODUCTION

Even at very low concentrations, trace elements (TEs) can profoundly affect life both positively and negatively.[1] Arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) are non-essential elements for human metabolism are listed in the top 20 hazardous substances by the US Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry.[2,3] Some heavy metals, such as zinc (Zn), chromium (Cr), copper (Cu) and nickel (Ni), are not only essential micronutrients, but also toxins that cause health hazards depending on their concentrations in the diet.[4,5] Zn deficiency affects human health worldwide, especially in developing countries.[6] In China, the rate of Zn deficiency is 3.9% for children aged 3–5 years.[7] There are various pathways for human exposure to TEs and the consumption of vegetables grown in TE-contaminated soil comprises one of the major pathways.[8] Therefore, the scientific assessment of TE uptake and accumulation characteristics of vegetables from the soil is necessary for the safe production of crops and evaluation of soil quality.[9,10]

As China has rapidly transitioned from a traditionally agricultural-based economy to an industrial and technology-based economy over the last 30 years, increasing contaminant levels are threatening agricultural soil, especially in South China where there is also increasing soil acidification.[11] This combination could heighten the risk of TE accumulation in food and subsequently endanger human health.[3,12] The TEs in crops mainly originate from the plant uptake of TEs from the soil, and so it is necessary to set scientific soil regulatory values to ensure the effective use of agricultural soil and food safety.[13,14] Although most countries have regulatory values for the total soil TE concentrations,[15,16] there is increasing interest in using a risk-based approach considering 'bioavailable' TE.[10,17] There are weak correlations between total soil TE concentrations and plant TE

* Correspondence to: B H Robinson, School of Physical and Chemical Science, University of Canterbury, Christchurch 8041, New Zealand. E-mail: brett.robinson@canterbury.ac.nz

a Institute of Quality Standard and Monitoring Technology for Agro-Products of Guangdong Academy of Agricultural Sciences, Guangzhou, China

b Key Laboratory of Testing and Evaluation for Agro-product Safety and Quality, Ministry of Agriculture and Rural Affairs, Guangzhou, China

c School of Physical and Chemical Sciences, University of Canterbury, Christchurch, New Zealand

concentrations because the TE uptake of plants from soil also depends on the plant variety and the available soil pool, which is affected by several soil properties, such as pH, organic matter content and cation exchange capacity.[18,19] Measuring the extractable TE concentration in soil may provide a more reliable prediction of TE accumulation in crops.[20]

Chinese flowering cabbage (*Brassica parachinensis* L.) is an important vegetable in China that is cultivated year-round and exported worldwide.[21,22] *B. parachinensis* has a higher bioaccumulation coefficient for Cd than other vegetables.[23,24] The Zn concentration in the dry matter of cabbage exceeds 50 mg kg^{-1},[25] which is higher than that in cereal crops by approximately 20 mg kg^{-1}.[26] Moreover, many *Brassica* species accumulate elevated concentrations of Ni, Pb and Cu,[27] which indicates that *B. parachinensis* is a sensitive indicator of TE contamination in soil.

In the present study, we hypothesized that a risk-based approach using a diluted salt to extract soil would provide a more robust assessment of TE uptake by *B. parachinensis* than the threshold for the total soil TE concentrations. We aimed to compare the current regulatory system based on total TE concentration and pH with a risk-based approach using a diluted neutral salt (0.01 mol L^{-1} $CaCl_2$) to estimate a bioavailable fraction, as suggested previously.[28,29] Because some TEs are both toxic and essential micronutrients for humans, the results of this study will provide a scientific basis for the safe utilization of Chinese farmland soil and for developing the nutritional value of *B. parachinensis*.

MATERIALS AND METHODS

Soil and vegetable sampling

Soil and vegetable samples were collected from the main production areas of *B. parachinensis* in several cities (Foshan, Huizhou, Guangzhou, Qingyuan, Zhaoqing, Zhongshan and Zhuhai) of Guangdong Province, South China (Fig. 1). These selected soils represented a range of TE concentrations, including sites suspected to be contaminated by their proximity to industry and areas with a history of sewage sludge application. Overall, 150 pairs of *B. parachinensis* samples and their corresponding growth soils were collected. At each sampling site with an area of 100–500 m^2, approximately 20 individual *B. parachinensis* plants were randomly sampled by removing the entire plant as a whole and then dividing it into the shoot and root. The corresponding soil was collected at a depth of 0–20 cm under the plant samples and mixed thoroughly.[30] All samples were stored in sealed polyethylene bags and transferred to the laboratory.[17]

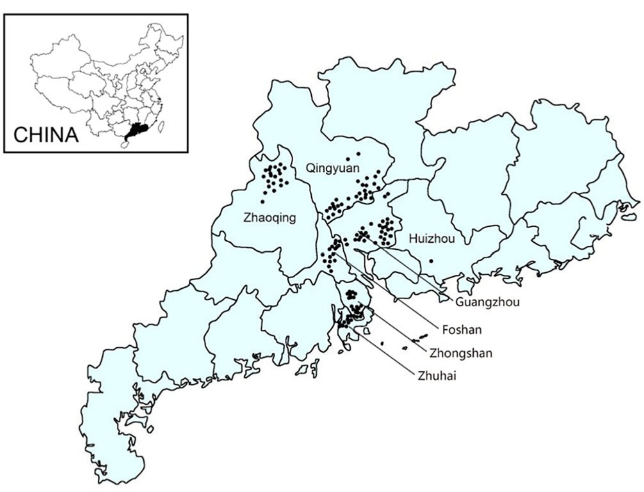

Figure 1. Distributions of the 150 sampling sites in different cities (Foshan, Huizhou, Guangzhou, Qingyuan, Zhaoqing, Zhongshan, Zhuhai) of Guangdong Province, China.

Soil analysis

The soil samples were air-dried at room temperature after removing stones and plant debris. Then, they were ground with a mortar and a pestle and passed through 2 mm (for pH and extractable TE analysis) and 0.149 mm (for organic matter and total TE concentration analysis) nylon screens.[31] The pH was measured using a soil suspension with soil water ratio of 1:2.5 and a glass pH electrode.[32] The soil organic matter content was determined by the Walkley Black method.[5] The total Cr, Cd, Pb, Ni, Cu and Zn concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700x ICPMS; Agilent Technologies, Santa Clara, CA, USA) based on the methodology from our previous work.[33] This was performed after acid digestion of 0.2 g soil for 4.5 h using a mixture of concentrated HNO_3 + $HClO_4$ + HF (6.4 + 1.6 + 4 mL) in a covered Teflon crucible with a temperature-controlled graphite digester (DigiBlock ED54; Lab-Tech, Shanghai, China) with the temperature gradually increased to 120 °C for 0.5 h, 150 °C for 0.5 h and 210 °C for 2.5 h. For the total As and Hg concentrations, 0.25 g of soil was added to colorimetric tubes with stoppers and digested with a mixture of concentrated $HCl:HNO_3:H_2O$ (3:1:4, 10 mL) in a water bath (100 °C) for 2 h and then subjected to atomic fluorescence spectrometry (AFS) (AFS-9130, Jitian Instruments Co. Ltd, Beijing, China).[9]

The available concentrations of Cr, Cd, Pb, Ni, Cu, Zn, As and Hg were determined by 10.0 g of soil with 100 mL of 0.01 mol L^{-1} $CaCl_2$ in centrifuge tubes.[34] Samples were placed on a horizontal shaker revolving at 180 times min^{-1} for 120 min in a room at a constant temperature of 20 ± 2 °C. After centrifugation at $1000 \times g$ for 10 min, the supernatant was filtered through 0.45-μm microporous barrel-type filters with Luer lock syringes and then diluted five times with 2% HNO_3 before analysis by ICP-MS.

Plant analysis

The vegetable samples were rinsed with tap and distilled water, and then divided into the edible parts (shoots) and roots to be homogenized with a blender (HR1848; Philips, Dongguan, China). After weighing, subsamples were digested with a mixture of $HNO_3:HClO_4$ (4:1, v/v) at 160 °C.[10] The Cr, Cd, Pb, Ni, Cu and Zn concentrations were subsequently measured by graphite furnace atomic absorption spectrometry (GFAAS; Zeenit 600; Analytik Jena AG, Jena, Germany), whereas the As and Hg concentrations were determined by AFS.[35]

Quality control/assurance

To ensure accuracy, the calibration curves were kept within the linear concentration range based on six working standard solutions (WAKO Pure Chemical Industries Ltd, Osaka, Japan), with regression coefficient (r^2) > 0.999.[35] Both blanks and standard reference materials (GSB-5 for vegetable samples, GSS-7 for soil samples, from the National Center for Standard Reference Materials of China, Guangzhou, China) were included for quality assurance and quality control.[10] Reproducibility was tested by re-analyzing 5% of the samples, and recovery was 90–107%. The limits of detection for Cr, Cd, Pb, Ni, Cu, Zn, As and Hg were 0.05, 0.002, 0.02, 0.2, 0.05, 0.5, 0.002 and 0.001 mg kg^{-1}, respectively. The limits of quantity were 0.2, 0.005, 0.005, 0.5, 0.2, 2.0, 0.005 and 0.003 mg kg^{-1}, respectively.

Statistical analysis

The soil TE concentrations were measured using dry matter in accordance with the methodology of the Soil Environmental Quality-Risk Control Standard for Soil Contamination of Agricultural Land.[36] In vegetables, it was measured using fresh matter, according to the National Food Safety Standard (FSS)-Contaminant Limit in Food set by the Chinese Ministry of Health.[37] All data were processed using Excel 2016 (Microsoft Corp., Redmond, WA, USA). Data were tested for normality and log-normally distributed data were log-transformed prior to statistical analyses. Differences between means were determined using analysis of variance (ANOVA) with a post-hoc Fisher's test. $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS, version 19.0 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

TE concentrations in soils and plants

The soil As, Cd, Cr, Cu, Hg, Ni, Pb and Zn concentrations are listed in Table 1. Soil had a mean pH value of 5.90 and soil organic matter content of 27.29 g kg^{-1}, which represents the typical characteristics of acidic soil in South China. The coefficient variations of the total TE concentrations in the soil for all eight TEs were over 44%, with the concentrations for most of the eight TEs ranging from low to moderately polluted levels according to the Chinese soil thresholds.[36] Therefore, soil selection was suitable for determining the validity of the Chinese soil regulations for the safe production of vegetables.[18] Note that these concentrations were not representative of soils throughout the region because we specifically chose soils with elevated TE concentrations. There were significant positive correlations between most of the total soil concentrations of the eight TEs (see Supporting information, Table S1). This suggested that a single factor, such as industrial activity, was the most important determinant of the total soil TE concentrations.[38]

Table 2 shows the TE concentrations in different parts of *B. parachinensis* and the percentage exceeding the Chinese FSS,[37] indicating that most vegetable samples posed no health threat even when grown in soils with elevated TE concentrations. Few samples exceeded FSS, including 2% for Cr, 3.33% for Cd and 1.33% for Pb. Thus, the current soil regulatory guidelines for *B. parachinensis* production are conservative because most plants growing in soils exceeding the guideline values had TE concentrations below FSS.[18] Table 2 also shows the root TE concentrations of *B. parachinensis*, which were much higher than those of the shoots. Although the roots of this vegetable are not routinely consumed, the results suggests that root vegetables such as radish, potato and carrot may contain higher TE concentrations.[39,40] Presently, there are no specialized soil environmental quality

Table 1. Overview of the TE concentrations in soil (mg kg^{-1} dry weight) and the percentages exceeding the Chinese soil threshold values[36] [total and extractable TE concentrations in soil are presented as averages and ranges (in brackets) ($n = 150$)]

Element	Total soil TEs	Coefficient variation (%)	Soil threshold[a]	Percentage exceeding soil threshold (%)	Extractable soil TEs
Cr	60.99 (10.08–333.13)	61.03	150/150/200/250	0.67	0.01 (0–1.08)
Cd	0.46 (0.06–5.69)	132.75	0.3/0.3/0.3/0.6	54.67	0.03 (0–0.79)
Pb	59.47 (20.30–406.03)	63.03	70/90/120/170	11.33	0.01 (0–0.34)
Ni	23.21 (5.57–49.29)	61.23	60/70/100/190	0	0.11 (0–2.51)
Cu	37.56 (7.88–99.29)	52.95	50/50/100/100	24.67	0.05 (0–0.44)
Zn	120.61 (42.03–357.21)	44.17	200/200/250/300	5.33	0.89 (0–12.97)
As	14.32 (1.90–45.37)	58.35	40/40/30/25	4.00	0.02 (0–0.18)
Hg	0.44 (0.05–11.76)	251.56	1.3/1.8/2.4/3.4	4.67	0

[a] The first value is at pH ≤ 5.5, the second value is 5.5 < pH ≤ 6.5, the third value is at 6.5 < pH ≤ 7.5 and the fourth value is at pH > 7.5.

Table 2. Overview of the TE concentrations in the edible parts (shoots) and roots of *Brassica parachinensis* (mg kg^{-1} fresh weight) and the percentages exceeding the Chinese food safety standards (FSS)[37] [plant TE concentrations are reported as averages and ranges (in brackets) ($n = 150$)]

Element	Edible parts	FSS	Percentage exceeding FSS (%)	Roots
Cr	0.13 (0.03–1.23)	0.5	2.00	0.47 (0–5.85)
Cd	0.05 (0.004–1.03)	0.2	3.33	0.06 (0.004–1.06)
Pb	0.08 (0.02–0.43)	0.3	1.33	0.29 (0–3.35)
Ni	0.08 (0.02–0.50)	NA	NA	0.30 (0–3.87)
Cu	0.49 (0.19–0.98)	NA	NA	0.52 (0.05–2.01)
Zn	3.64 (1.35–10.57)	NA	NA	6.41 (1.69–33.06)
As	0.01 (0.003–0.06)	0.5	0	0.07 (0–0.71)
Hg	0.001 (0–0.02)	0.01	0	0.001 (0–0.01)

NA, no data for analysis.

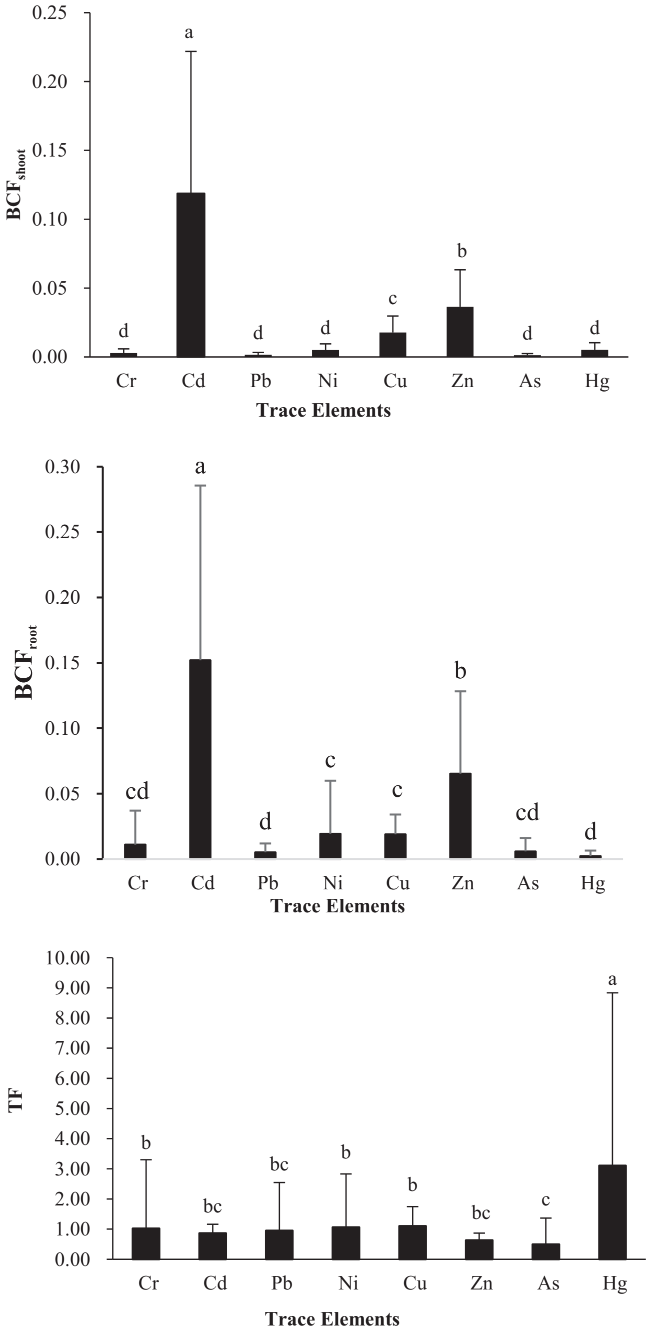

Figure 2. The bioconcentration factor (BCF) for shoots (a) and roots (b) and transfer coefficient (TF) (c) of *Brassica parachinensis* for the eight trace elements. Bars with the same letter are not significantly different.

standards for specific vegetables in China. Therefore, developing crop-specific soil guidelines for different types of crops is necessary in future research.

Plant uptake capacity of different TEs

Among the many soil–plant transfer indices used to evaluate the environmental safety risks of soil TEs, the bioconcentration factor (BCF) and transfer coefficient (TF) are two of the most essential.[11,14] BCF is the ratio of TE concentration in different parts of the plant to that in the soil, and it determines the degree of plant TE uptake from the soil.[3,9] TF is the ratio of TE in the aboveground part to that in the underground part, which is an important indicator for measuring the distribution and transportation of TEs in plants.[17] Higher values of BCF and TE indicate the greater potential of plants to transfer TEs from the soil to the food chain.[17] The TE uptake capacity of *B. parachinensis* demonstrates obvious differences (Fig. 2). Generally, the uptake capacity of the shoots of *B. parachinensis* was Cd > Zn > Cu > Hg > Ni > Cr > Pb > As. The BCF$_{shoot}$ (0.119) of Cd was significantly higher than that of other TEs, followed by Zn (0.036) and Cu (0.018). For BCF$_{root}$, the order of the eight TEs was Cd > Zn > Ni = Cu > Cr > As > Pb > Hg. The BCF$_{root}$ (0.152) of Cd was significantly higher than that of other TEs, followed by Zn (0.065), Ni (0.019) and Cu (0.019). TEs had different transfer rates from belowground to aboveground plant parts, in the order Hg > Cu > Ni > Cr > Pb > Cd > Zn > As. The TF values of Hg, Cu, Ni and Cr were over 1, with that of Hg being the largest (3.105). Even at extremely low concentrations, long-term exposure of Hg can cause serious health problems for humans.[41,42] Therefore, it is necessary to avoid planting leafy vegetables including *B. parachinensis* in Hg-contaminated soil.

Compared with other elements, Cd was more easily absorbed by the shoots and roots of *B. parachinensis*, whereas As was less easily accumulated in the shoots. This was consistent with the finding of Chang *et al.*[23] that Cd had a high transfer capacity from soil into vegetables, with a BCF value approximately 30 times that of Hg and 50 times that of Cr, Pb and As. The mobility of Cd in the soil and its bioaccumulation in the food chain surpass those of all other heavy metals, especially for vegetables grown in acidic soils of South China, which are prone to Cd accumulation.[20,43,44] This may be caused by competition between Cd^{2+} and Ca^{2+} because Ca^{2+} has an ionic radii and valance more similar to Cd^{2+} than to other TEs.[23,45] The BCF and TF of Zn and Cu in *B. parachinensis* were relatively greater than those of most other TEs. The higher uptake of essential metals such as Cu, Zn and Ni observed in other vegetables may be a result of specific uptake transporters that maintain their growth.[46] Li *et al.*[47] compared the Zn concentrations of several leaf vegetables and found that *B. parachinensis* took up more Zn (3.3 mg kg^{-1}) than other vegetables. Both Zn and Cu are essential elements for human health, although they may be toxic when their concentrations exceed safe limits.[48] Although, generally, there is little risk of dietary copper deficiency in healthy people, there are numerous factors that increase the risk of copper deficiency.[4] Additionally, approximately 0.45 million children under 5 years of age die of Zn deficiency and over 2 billion people suffer from it.[49] Because dietary diversification is one of the common approaches to alleviate micronutrient deficiency,[4,50] the inclusion of *B. parachinensis* grown in biofortified soil in the diet may be a potential resource for ameliorating Zn and Cu deficiency.

Plant TE uptake as a function of the total soil TE concentrations

The correlations between the soil and shoot TE concentrations are shown in Table 3. There were significant positive correlations between the total concentrations in soil and shoots for Cr, Cd, Ni and As, whereas no significant correlations existed for Pb, Cu, Zn and Hg. Although the total soil TE is the most important source for vegetable absorption, there are other TE sources that influence TE accumulation in leafy vegetables, such as the application of pesticides and chemical fertilizers (e.g. Cu and Zn) and atmospheric deposition (e.g. Pb and Hg).[11,51] Additionally, the processes of TE uptake accumulation, and redistribution in

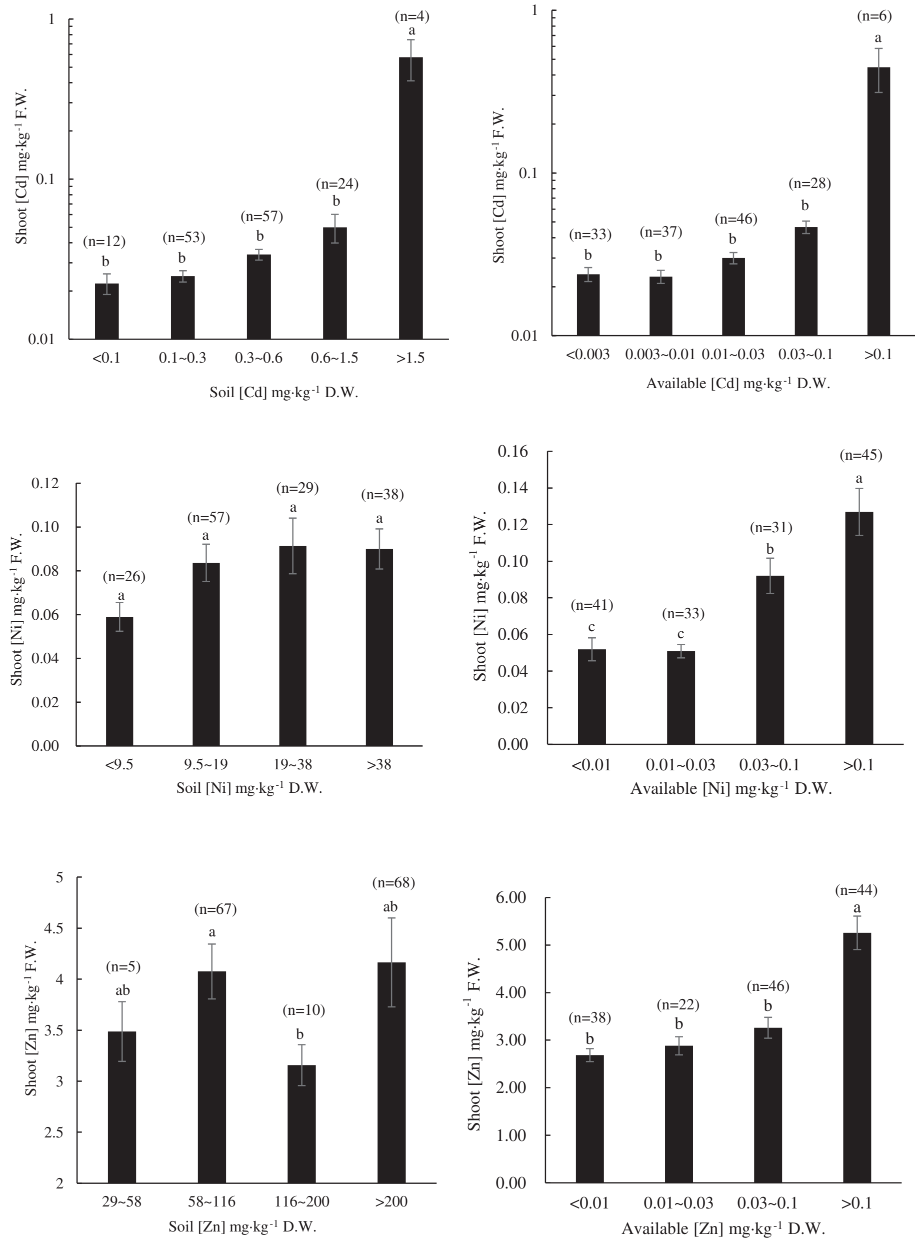

Figure 3. The shoot trace element (TE) concentrations of *Brassica parachinensis* with the increasing soil TE concentration bands. For the total TEs, the concentration bands represent (from low to high) the background concentration or twice the background concentration, to the 3 or 4 times the soil threshold values used in Chinese regulations. For the available TEs, the concentration bands were set mainly based on the distribution ranges and the sample numbers. D.W., dry weight. Bars with the same letter are not significantly different.

vegetables are dependent on plant cultivars and the bioavailable fraction of soil TE, which are notably affected by several soil properties, especially pH, organic carbon content, clay content and soil redox potential.[52,53]

Figure 3 shows the shoot TE concentrations with increasing concentration bands in the soil. The total TE concentration bands were divided into the soil background concentration at 3–4 times the Chinese regulatory thresholds. For Cd, plant concentration was significantly increased in the highest soil concentration band (>1.5 mg kg^{-1}), whereas the other TEs showed no significant differences with respect to their shoot concentrations among the different total soil concentration bands. Based on the multiple linear regression analysis and curve estimation of Cd concentrations for 276 and 170 paired soil–vegetable samples excluding pH, the calculated threshold values of the total soil Cd concentrations were 2.42 mg kg^{-1} and 1.94 mg kg^{-1} for leafy vegetables and *B. chinensis* in Guangdong Province, respectively.[10,30] Xiao *et al.*[18] proposed a threshold value of 0.84 mg kg^{-1} for *B. chinensis* with 31 paired soil–vegetable samples in Zhejiang Province, South China. Considering different soil pH levels ($\text{pH} < 5.0$, $5.5 \leq \text{pH} \leq 6.5$, $6.5 < \text{pH} \leq 7.5$, $\text{pH} > 7.5$), Hu *et al.*[17] computed the threshold values of the total soil Cd concentrations for leafy vegetables as 0.32, 0.48, 1.56 and 2.64 mg kg^{-1}, respectively. Most of the proposed threshold values were much higher than the current Chinese threshold values for total Cd in agricultural soil (0.3 mg kg^{-1} for pH ≤ 7.5 and 0.6 mg kg^{-1} for $\text{pH} > 7.5$).[36] This suggests that the current Cd soil threshold values might be too strict for the safe production of leafy vegetables, which may result in large amounts of soil that could produce safe vegetables being considered as contaminated and excluded from agricultural production.[18] It also indicates that further studies focusing on different crops and agricultural regions are necessary to provide accurate and rigorous soil TE regulation for vegetable cultivation.

Plant TE uptake as a function of the extractable soil TE concentrations

The correlations between the plant TE concentrations and the $CaCl_2$-extractable concentrations in soil are shown in Table 3. For Cr, Pb, Cu and As, there were no significant correlations. To a small extent, this may be related to the multiple sources of TEs taken up by plants other from agricultural soil,[51,54] along with the influence of physiological, anatomical and biochemical factors of different crop cultivars.[3,55] For Hg, the concentrations were below the detection limit (< 0.002 mg kg^{-1}). Significantly positive correlations were obtained for Cd, Ni and Zn, and their correlation coefficients for extractable concentrations were much greater than those for the total concentrations, indicating that the extractable concentrations provided a better indication of plant uptake for Cd, Ni and Zn. Similar observations were made by Yang *et al.*[53] for the correlations of $CaCl_2$-Cd in soil and Cd in vegetables and Sun *et al.*[30] for the correlations of DTPA-Cd in soil and Cd in vegetables.

Dividing the extractable TE concentrations into bands based on the distribution ranges and sample numbers showed significant differences in plant uptake for Cd, Ni and Zn (Fig. 3). The other elements without significant differences for their TE concentrations in shoots among the different total soil or extractable TE concentration bands were not shown in the figures. Plant uptake was significantly increased for $CaCl_2$-extractable Cd (> 0.1 mg kg^{-1}), Ni (> 0.03 mg kg^{-1}) and Zn (> 0.1 mg kg^{-1}), which are the thresholds affecting TE concentrations in *B. parachinensis*. The proposed extractable soil Cd thresholds with DTPA extraction were calculated as 0.13–0.37 mg kg^{-1} for different leafy vegetables in Zhejiang Province[18] and as 1.08 mg kg^{-1} for leafy vegetables[30] and 1.43 mg kg^{-1} for *B. chinensis*[10] in Guangdong Province. Although the established soil quality TE thresholds for most countries are derived from the total TE concentrations with or without involving pH,[16,44] some countries such as Japan, Switzerland and Germany use the extractable TE concentrations.[10,56] The soil thresholds based on the available TEs are generally more reliable for food safety than those based on the total TEs.[31,43] Because $CaCl_2$ (0.01 mol L^{-1}) simulates the ionic strength of the soil solution and is widely used to extract the readily bioavailable TEs from soil,[57] the $CaCl_2$-extractable TE threshold is considered as a good indicator of soil TE bioavailability, especially for Pb and Cd.[31,58] The most important thing to set guidelines for $CaCl_2$-extractable TEs of agricultural soil in future is development of robust extraction and detection methods.

Table 3. Correlation coefficients ($n = 150$, except Hg) for the shoot TE concentrations of *Brassica parachinensis versus* the total and $CaCl_2$-extractable TE concentrations in soil

Element	Total soil TE	Extractable soil TE
Cr	0.22**	0.01^{NS}
Cd	0.52**	0.59**
Pb	0.03^{NS}	0.03^{NS}
Ni	0.16*	0.63**
Cu	0.08^{NS}	0.01^{NS}
Zn	−0.12^{NS}	0.49**
As	0.24**	0.09^{NS}
Hg	0.16^{NS}	NA

Statistical significance: *$p < 0.05$ and **$p < 0.01$. NS, no significant correlation. NA, no data for analysis.

Correlation analysis of soil TE concentrations, soil pH and organic matter content

There were significant positive correlations $0.22 < r^2 < 0.44$ between the total and extractable elements for Cr, Cd, Pb, Ni, Cu and As (Table 4), which indicates that the extractable concentrations were likely influenced by the total concentrations. Most total TEs demonstrated no correlation or a weak positive correlation with soil pH. However, most of the $CaCl_2$-extractable TEs were significantly negatively correlated with soil pH, especially Cd, Ni and Zn. Soil pH is commonly considered a major factor in extractable soil TE concentrations and vegetable TE accumulation because it controls metal speciation, solubility and mobility in the soil metal pool, and a low soil pH usually facilitates TE bioavailability.[9,54] Taking the total TE concentrations as the soil thresholds, soil pH significantly contributed to the regression model fit for Cd transfer in soil-crop systems.[28,59] The total soil TEs should be combined with soil pH to be reasonably predictive of TE concentrations in aboveground plant tissue. As the pH effect is already accounted for when using the $CaCl_2$ extractable TEs to predict plant TE accumulation,[31] pH has no added value for calculating soil $CaCl_2$-extractable TE thresholds for *B. parachinensis* production, which would improve the accuracy and convenience of the soil environmental quality guideline.[30,60]

Table 4. Correlation coefficients among total TE concentrations, extractable TE concentrations, soil pH and organic matter content

Element	Total-extractable TE	Total TE – pH	Total TE – organic matter	Extractable TE – pH	Extractable TE – organic matter
Cr	0.44**	0.17*	0.10^{NS}	−0.06^{NS}	0.00^{NS}
Cd	0.38**	0.17*	0.10^{NS}	−0.71**	−0.24**
Pb	0.35**	0.09^{NS}	0.41**	−0.31*	0.10^{NS}
Ni	0.22**	0.09^{NS}	0.09^{NS}	−0.64**	−0.16^{NS}
Cu	0.44**	0.11^{NS}	0.16*	−0.25**	0.26**
Zn	−0.07^{NS}	0.32**	0.29**	−0.78**	−0.05^{NS}
As	0.41**	0.03^{NS}	0.12^{NS}	−0.14^{NS}	0.29**
Hg	NA	0.08^{NS}	0.42**	NA	NA

Statistical significance: *$p < 0.05$ and **$p < 0.01$. NS, no significant correlation. NA, no data for analysis.

Soil organic matter content is another important parameter that affects the TE availability and mobility in soil crop systems. Sometimes, the impaction depends on other soil traits, TE types, and crop cultivars.[43,61] In the present study, soil organic matter was positively correlated with the total soil Pb, Cu, Zn and Hg concentrations, whereas there were weak and inconsistent correlations between soil organic matter and extractable TE concentrations. TEs bind to soil organic matter, both as complexes in soil solution and organic matter associated with the soil matrix.[62] TEs that are complexed by organic matter, either in the soil matrix or soil solution, are less available for plant uptake.[54,59] Therefore, in terms of plant uptake, the effect of the higher TE concentrations in the organic matter-rich soils were likely offset by a reduction in plant-availability caused by the organic matter itself.

CONCLUSIONS

In general, the current soil TE regulatory guidelines for the safe production of *B. parachinensis* are conservative and provide decent food chain protection. After comparing the correlation coefficients for the shoot TE concentrations and the total soil and extractable TE concentrations, we suggest that the extractable TE concentrations would provide a better indication of plant uptake than the total concentrations, especially for Cd, Ni and Zn. Dividing the 150 paired vegetable–soil samples into several bands according to the different total and extractable soil TE concentration values, plant concentration was significantly increased only above the highest band value for total soil Cd (1.5 mg kg^{-1}), and the extractable Cd (0.1 mg kg^{-1}), Ni (0.03 mg kg^{-1}) and Zn (0.1 mg kg^{-1}), which would be the thresholds affecting the TE concentration in *B. parachinensis*. To promote effective soil use for ensuring the safe production of vegetables in China, future research could focus on providing crop-specific guidelines for individual vegetables and developing effective detection methods for soil quality thresholds based on the available TE concentrations. Additionally, as a result of the relatively greater BCF for Zn and TF for Cu, *B. parachinensis* may be a potential resource of Zn and Cu in the human diet. The present study provides important insights into soil quality measurement and vegetable TE uptake, and indicates that a plant-specific risk analysis accounting for variations in soil factors could be more useful in providing an effective risk-based approach for the safe production of vegetables and the management of TE-contaminated soils.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China Youth Fund (No. 31800432), the National Key R&D Program of China (No. 2019YFC1605602), the National Overseas Study for Senior Research Scholar, Visiting Scholar and Postdoctoral Program (CSC No. 201808440042), and the Science and Technology Major Project of Guangxi (grant number AA20161002).

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Fraga CG, Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspects Med* **26**:235–244 (2005).

2 ATSDR T, ATSDR (Agency for Toxic Substances and Disease Registry). Prepared by Clement International Corp under contract 205,88–0608. 2017.

3 Setia R, Dhaliwal SS, Singh R, Kumar V, Taneja S, Kukal SS *et al.*, Phytoavailability and human risk assessment of heavy metals in soils and food crops around Sutlej river, India. *Chemosphere* **263**:128321 (2021).

4 Prohaska JR, Impact of copper deficiency in humans. *Ann N Y Acad Sci* **1314**:1–5 (2014).

5 Pandit P, Mangala P, Saini A, Bangotra P, Kumar V, Mehra R *et al.*, Radiological and pollution risk assessments of terrestrial radionuclides and heavy metals in a mineralized zone of the siwalik region (India). *Chemosphere* **254**:126857 (2020).

6 Zhang CM, Zhao WY, Gao AX, Su TT, Wang YK, Zhang YQ *et al.*, How could agronomic biofortification of rice be an alternative strategy with higher cost-effectiveness for human iron and zinc deficiency in China? *Food Nutr Bull* **39**:246–259 (2018).

7 Bi Y, Yang ZY, Pang XH, Duan YF, Lai JQ, Jiang S, Wang J, Zhang HM, The phenomenon of zinc deficiency among children in China aged 3–5 years old. Proceedings of the Nutrition Society, eds Dr Janice E. Drew Rowett, Institute of Nutrition and Health, University of Aberdeen, UK. Cambridge University Press. 10 June 2020.

8 Huang Y, Wang L, Wang W, Li T, He Z and Yang X, Current status of agricultural soil pollution by heavy metals in China: a meta-analysis. *Sci Total Environ* **651**:3034–3042 (2019).

9 Wu ST, Zheng YW, Li XB, Han YX, Qu MH, Ni ZL *et al.*, Risk assessment and prediction for toxic heavy metals in chestnut and growth soil from China. *J Sci Food Agric* **99**:4114–4122 (2019).

10 Li FR, Wen D, Wang FH, Sun FF, Wang X, Du YQ *et al.*, Derivation of soil Pb/cd/as thresholds for safety of vegetable planting: a case study for pakchoi in Guangdong Province, China. *J Integr Agric* **18**:179–189 (2019).

11 Qin GW, Niu ZD, Yu JD, Li ZH, Ma JY and Xiang P, Soil heavy metal pollution and food safety in China: effect, sources and removing technology. *Chemosphere* **267**:129205 (2021).

12 Zeng LP, Zhou F, Zhang XC, Qin JH and Li HS, Distribution of heavy metals in soils and vegetables and health risk assessment in the vicinity of three contaminated sites in Guangdong Province, China. *Hum Ecol Risk Assess* **24**:1901–1915 (2018).

13 Chen SB, Wang M, Li SS, Zhao ZQ and Wen-di E, Overview on current criteria for heavy metals and its hint for the revision of soil environmental quality standards in China. *J Integr Agric* **17**:765–774 (2018).

14 Rai PK, Lee SS, Zhang M, Tsang YF and Kim KH, Heavy metals in food crops: health risks, fate, mechanisms, and management. *Environ Int* **125**:365–385 (2019).

15 Moreno JL, Bastida F, Ros M, Hernández T and García C, Soil organic carbon buffers heavy metal contamination on semiarid soils: effects of different metal threshold levels on soil microbial activity. *Eur J Soil Biol* **45**:220–228 (2009).

16 Jennings AA, Analysis of worldwide regulatory guidance values for the most commonly regulated elemental surface soil contamination. *J Environ Manage* **118**:72–95 (2013).

17 Hu WY, Huang B, Borggaard OK, Ye M, Tian K, Zhang HD *et al.*, Soil threshold values for cadmium based on paired soil-vegetable content analyses of greenhouse vegetable production systems in China: implications for safe food production. *Environ Pollut* **241**: 922–929 (2018).

18 Xiao WD, Ye XZ, Zhang Q, Chen D, Hu J and Gao N, Evaluation of cadmium transfer from soil to leafy vegetables: influencing factors, transfer models, and indication of soil threshold contents. *Ecotoxicol Environ Saf* **164**:355–362 (2018).

19 Wu JH, Song QM, Zhou JY, Wu YX, Liu XW, Liu JJ *et al.*, Cadmium threshold for acidic and multi-metal contaminated soil according to *Oryza sativa* L. cadmium accumulation: influential factors and prediction model. *Ecotoxicol Environ Saf* **208**:111420 (2021).

20 Wang P, Chen H, Kopittke PM and Zhao FJ, Cadmium contamination in agricultural soils of China and the impact on food safety. *Environ Pollut* **249**:1038–1048 (2019).

21 Akram W, Ahmad A, Guo JX, Yasin NA, Akbar M, Luo WL *et al.*, Occurrence of head rot disease caused by *Fusarium verticillioides* on Chinese flowering cabbage (*Brassica rapa* L *subsp. parachinensis*) in China. *Crop Protect* **134**:105180 (2020).

22 Zhao HM, Du H, Xiang L, Chen YL, Lu LA, Li YW *et al.*, Variations in phthalate ester (PAE) accumulation and their formation mechanism in Chinese flowering cabbage (*Brassica parachinensis* L.) cultivars grown on PAE-contaminated soils. *Environ Pollut* **206**:95–103 (2015).

23 Chang CY, Yu HY, Chen JJ, Li FB, Zhang HH and Liu CP, Accumulation of heavy metals in leaf vegetables from agricultural soils and

associated potential health risks in the Pearl River Delta, South China. *Environ Monit Assess* **186**:1547–1560 (2014).
24 Li Q, Cai S, Mo C, Chu B, Peng LH and Yang FB, Toxic effects of heavy metals and their accumulation in vegetables grown in a saline soil. *Ecotoxicol Environ Saf* **73**:84–88 (2010).
25 Zu YQ, Li Y, Bock L, Schvartz C and Colinet G, Soil effects on the cd and Zn contents of Chinese cabbage in Yunnan Province, China. *Arch Agron Soil Sci* **56**:107–117 (2010).
26 Wissuwa M, Ismail AM and Graham RD, Rice grain zinc concentrations as affected by genotype, native soil-zinc availability, and zinc fertilization. *Plant and Soil* **306**:37–48 (2008).
27 Mourato M, Moreira I, Leitão I, Pinto F, Sales J and Martins L, Effect of heavy metals in plants of the genus *Brassica*. *Int J Mol Sci* **16**: 17975–17998 (2015).
28 Zhang MK, Liu ZY and Wang H, Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. *Commun Soil Sci Plant Anal* **41**:820–831 (2010).
29 Dai YC, Nasir M, Zhang YL, Wu HM, Guo HH and Lv JL, Comparison of DGT with traditional methods for assessing cadmium bioavailability to *Brassica chinensis* in different soils. *Sci Rep* **14206**:1–9 (2017).
30 Sun FF, Wang FH, Wang X, He W, Wen D, Wang QF *et al*., Soil threshold values of total and available cadmium for vegetable growing based on field data in Guangdong Province, South China. *J Sci Food Agric* **93**:1967–1973 (2013).
31 Tian K, Xing Z, Liu GM, Wang HF, Jia MM, Hu WY *et al*., Cadmium phytoavailability under greenhouse vegetable production system measured by diffusive gradients in thin films (DGT) and its implications for the soil threshold. *Environ Pollut* **241**:412–421 (2018).
32 Huang CQ, Zhao W, Liu F, Tan WF and Koopal LK, Environmental significance of mineral weathering and pedogenesis of loess on the southernmost loess plateau, China. *Geoderma* **163**:219–226 (2011).
33 Wen D, Yan D, Zhao PH, Li L and Du RY, Fast determination of 14 elements in China soil pollution survey with high throughput full digestion method by ICPMS. *Environ Chem* **37**:1432–1435 (2018).
34 Kandziora-Ciupa M, Ciepal R and Nadgórska-Socha A, Assessment of heavy metals contamination and enzymatic activity in pine forest soils under different levels of anthropogenic stress. *Pol J Environ Stud* **25**:1045–1051 (2016).
35 Hu YA, Liu XP, Bai JM, Shih KM, Zeng EY and Cheng HF, Assessing heavy metal pollution in the surface soils of a region that had undergone three decades of intense industrialization and urbanization. *Environ Sci Pollut Res* **20**:6150–6159 (2013).
36 National Environmental Protection Agency of China, 2018. Soil environmental quality: Risk control standard for soil contamination of agricultural land (GB 15618–2018).
37 National Health Agency of China, 2017. Maximum Levels of Contaminants in Foods (GB 2762–2017).
38 Wang YT and Björn LO, Heavy metal pollution in Guangdong Province, China, and the strategies to manage the situation. *Front Environ Sci* **2**:9 (2014).
39 Wang Y, Shen H, Zhu XW, Li C, Zhang W, Xie Y *et al*., Transport, ultrastructural localization, and distribution of chemical forms of lead in radish (*Raphanus sativus* L.). *Front Plant Sci* **6**:1–13 (2015).
40 Liang H, Wu WL, Zhang YH, Zhou SJ, Long CY, Wen J *et al*., Levels, temporal trend and health risk assessment of five heavy metals in fresh vegetables marketed in Guangdong Province of China during 2014–2017. *Food Control* **92**:107–120 (2018).
41 Genchi G, Sinicropi MS, Carocci A, Lauria G and Catalano A, Mercury exposure and heart diseases. *Int J Environ Res Public Health* **14**:733 (2017).
42 Khanam R, Kumar A, Nayak AK, Shahid M, Tripathi R, Vijayakumar S *et al*., Metal(loid)s (as, hg, se, Pb and cd) in paddy soil: bioavailability and potential risk to human health. *Sci Total Environ* **699**:134330 (2020).
43 Rafiq MT, Aziz R, Yang X, Xiao W, Stoffella PJ, Saghir A *et al*., 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. *PLoS One* **9**:1–9 (2014).
44 Yang Y, Chen WP, Wang ME and Peng C, Regional accumulation characteristics of cadmium in vegetables: influencing factors, transfer model and indication of soil threshold content. *Environ Pollut* **219**: 1036–1043 (2016).
45 Kim YY, Yang YY and Lee Y, Pb and cd uptake in rice roots. *Physiol Plant* **116**:368–372 (2002).
46 Fu XZ, Zhou X, Xing F, Ling LL and Peng LZ, Genome-wide identification, cloning and functional analysis of the zinc/iron-regulated transporter-like protein ZIP gene family in trifoliate orange (*Poncirus trifoliata* L. Raf.). *Front Plant Sci* **19**:588–589 (2017).
47 Li QS, Chen Y, Fu HB, Cui ZH, Shi L, Wang LL *et al*., Health risk of heavy metals in food crops grown on reclaimed tidal flat soil in the Pearl River estuary, China. *J Hazard Mater* **227–228**:148–154 (2012).
48 Kisa D, Öztürk L, Doker S and Gökçe İ, Expression analysis of metallothioneins and mineral contents in tomato (*Lycopersicon esculentum*) under heavy metal stress. *J Sci Food Agric* **97**:1916–1923 (2017).
49 Stein AJ, Global impacts of human mineral malnutrition. *Plant Soil* **335**: 133–154 (2010).
50 Yuan L, Wu LH, Yang CL and Lv Q, Effects of iron and zinc foliar applications on rice plants and their grain accumulation and grain nutritional quality. *J Sci Food Agric* **93**:254–261 (2013).
51 Li X, Zhang J, Gong Y, Liu Q, Yang S, Ma J *et al*., Status of copper accumulation in agricultural soils across China (1985–2016). *Chemosphere* **244**:125516 (2020).
52 Chen HY, Yuan XY, Li TY, Hu S, Ji JF and Wang C, Characteristics of heavy metal transfer and their influencing factors in different soil–crop systems of the industrialization region, China. *Ecotoxicol Environ Saf* **126**:193–201 (2016).
53 Yang Y, Chen WP, Wang ME, Li YL and Peng C, Evaluating the potential health risk of toxic trace elements in vegetables: accounting for variations in soil factors. *Sci Total Environ* **584–585**:942–949 (2017).
54 Zhang S, Song J, Cheng YW, Christie P, Long J and Liu LF, Derivation of reliable empirical models describing lead transfer from metal-polluted soils to radish (*Raphanus sativa* L.): determining factors and soil criteria. *Sci Total Environ* **613–614**:72–80 (2018).
55 Ding CF, Li XG, Zhang TL, Ma YB and Wang XX, Phytotoxicity and accumulation of chromium in carrot plants and the derivation of soil thresholds for Chinese soils. *Ecotoxicol Environ Saf* **108**:179–186 (2014).
56 Smolders E, Oorts K, Sprang PV, Schoeters I, Janssen CR, McGrath SP *et al*., Toxicity of trace metals in soil as affected by soil type and aging after contamination: using calibrated bioavailability models to set ecological soil standards. *Environ Toxicol Chem* **28**: 1633–1642 (2009).
57 Xu QT and Zhang MK, Source identification and exchangeability of heavy metals accumulated in vegetable soils in the coastal plain of eastern Zhejiang province, China. *Ecotoxicol Environ Saf* **142**: 410–416 (2017).
58 Peng M, Zhao C, Ma H, Yang Z, Yang K, Liu F *et al*., Heavy metal and Pb isotopic compositions of soil and maize from a major agricultural area in Northeast China: contamination assessment and source apportionment. *J Geochem Explorat* **208**:106403 (2020).
59 Song NN, Wang FL, Ma YB and Tang SR, Using DGT to assess cadmium bioavailability to ryegrass as influenced by soil properties. *Pedosphere* **25**:825–833 (2015).
60 Ding CF, Ma YB, Li XG, Zhang TL and Wang XG, Derivation of soil thresholds for lead applying species sensitivity distribution: a case study for root vegetables. *J Hazard Mater* **303**:21–27 (2016).
61 Liu P, Ptacek CJ and Blowes DW, Mercury complexation with dissolved organic matter released from thirty-six types of biochar. *Bull Environ Contam Toxicol* **103**:1–6 (2018).
62 Robinson BH, Bañuelos G, Conesa HM, Evangelou MWH and Schulin R, The phytomanagement of trace elements in soil. *Crit Rev Plant Sci* **28**:240–266 (2009).

