Original Research

Variations in Cd and Pb Accumulations of Hot Pepper (*Capsicum annuum* L.) Cultivars for Screening Pollution- and Nitrate-Safe Cultivars

Li Chen[1], Zheng-Xu Cui[2], Dan Wang[1, 2]*

[1]College of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, China
[2]State Defense Key Laboratory of Nuclear Waste and Environmental Security, Southwest University of Science and Technology, Mianyang, China

Abstract

Screening pollution-safe cultivars has become one of the most direct and cost-effective methods for reducing metals entering the food chain. The aims of this study were to screen low Cd and Pb hot pepper cultivars and clarify the mechanisms of low Cd and Pb accumulation in fruits. In this study, we found that fruit Cd and Pb concentrations were significantly different ($p<0.05$) among cultivars and were also significantly ($p<0.05$) affected by Cd and Pb levels. Meanwhile, fruit Cd and Pb concentrations are significantly positively correlated with both the shoot Cd and Pb concentrations and the translocation factors. Nevertheless, there were no significant ($p<0.05$) correlations between the fruit Cd and Pb concentrations and the root Cd and Pb concentrations. Two hot pepper cultivars, Nos. 8 (Youma) and 11 (Xianglaerjintiao), were identified as low Cd and Pb cultivars. The nitrate content of No. 11 (Xianglaerjintiao) exceeded 440 mg·kg⁻¹ FW in T2 treatment, thus the No. 8 (Youma) can be grown to increase agricultural food safety compared to the No. 11 (Xianglaerjintiao). Therefore, the No. 8 (Youma) is suitable to be planted in low Cd- and Pb-contaminated soils for screening pollution-safe cultivars.

Introduction

Heavy metals (HMs) pollution in agricultural soil has become a worldwide environmental problem, because the HMs easily accumulate in soil and pose a great threat to the ecosystem, agro-system, and people's health [1-3]. Among HM-contaminated soils, cadmium (Cd) and lead (Pb) contamination in soils have been a worldwide environmental problem, especially in China [4], owing to exploitation and smelting of mines, deposition, sewage discharge, and the excessive use of fertilizers [5]. For example, Cd and Pb in the percentage

*e-mail: wangdanxkd@163.com

of soil samples (7.0% and 1.5%) exceed the Ministry of Environmental Protection limit in China [4]. Crops are the main source of Cd and Pb entering the human body. Therefore, screening low heavy metals (Cd and Pb) levels in the edible part of crops is a practical technique for reducing the health risks of heavy metal-contaminated soil.

The remediation contaminated by HMs has been reported by many researchers, such as adsorption, leaching, and electrokinetic remediation [6-8]. However, these techniques have some disadvantages in practice such as slow process, high cost, and damage of soil structure. The cycle of phytoextraction is also too long to quickly amend many metals-polluted soils. Furthermore, it is difficult for farmers to fallow the farmland for soil remediation, because the demand of farmers to produce food is quite high, especially in China. Thus, the screening crop cultivars of low HMs have been proposed by several researchers, which is an effective method to reduce ecological risks of heavy metals-contaminated soils [9, 10]. In past years, researchers found that the accumulation and distribution of HMs (including Cd and Pb) varied largely not only among crop species but also among cultivars [11, 12]. Many low Cd and Pb accumulation cultivars were selected from different crops such as Chinese cabbage [12], maize [13], wheat [15], pakchoi [16], and so on. Based on these studies, the investigations of pollution-safe crop cultivars in HM-contaminated soils have been a concern of researchers [17, 18].

At present, many researchers have reported that the transport processes mediating Cd and Pb accumulation in phytoremediation, which includes the absorption of Cd and Pb by roots, Cd and Pb translocation from the root to the aboveground parts, and xylem-to-phloem Cd and Pb transfer [19-21]. In addition, a few portions of Cd and Pb are translocated from the roots to the edible plant parts, for example fruits. The Cd and Pb levels of edible plant parts may be influenced by several physiological and biochemical features of the different species. Liu et al. [13] found that the edible parts of Lvxing 70 and Dongchu have the lowest and highest Cd accumulation properties in the 12 cabbage genotypes, respectively. Liu et al. [15] have also confirmed that wheat cultivar LF-13 showed the lowest Cd and Pb accumulation characteristics in their shoots among 30 wheat cultivars. Meanwhile, vegetables are a crop that is accumulated to nitrates easily, which may be related to their nutritional properties [22]. The study showed that 72-94% nitrates entering the human body are derived from vegetables, and excess nitrate content causes adverse effects on the human body [23, 24]. Therefore, the nitrate content of vegetables has become an important indicator to measure the health and safety of vegetables.

Hot pepper, a fruit vegetable with a more than 9000-year history, is widely cultivated in southern China. In 2015, the cultivated area in China is about 1.47 million hm^2, with 28 million tons of production.

Previous studies have shown that hot pepper can easily absorb and accumulate HMs (including Pb and Cd) from soils, which may cause adverse health effects [25]. Therefore, the screening of hot pepper cultivars with low Cd and Pb accumulation can reuse the contaminated soils and produce safe hot peppers in southern China.

Agricultural land has been widely polluted by HMs due to industrial activities, municipal sewage irrigation, and waste disposal practices in the industrial city of China. Thus, the aim of this study was to reduce the agricultural food risks through screening low accumulation cultivars in the Cd- and Pb-contaminated soil. The objectives were to: (1) determine the variety of biomass and nitrate content in hot pepper fruits, (2) screen low Cd and Pb concentrations from the tested the edible parts of hot pepper cultivars, and (3) investigate the differences in the uptake, translocation, and accumulation of Cd and Pb in hot pepper cultivars to clarify the mechanisms of low Cd and Pb accumulation in hot pepper fruits.

Materials and Methods

Hot Pepper and Soil Materials

Hot pepper (*Capsicum annuum* L.) cultivar seeds (Table 1) were purchased from the agricultural wholesale market of Fucheng District (Mianyang City, Sichuan Province, China). The soil samples (yellow earth) were selected from the topsoil (0-24 cm) in Longshan Garden of the Southwest University of Science and Technology. The basic physicochemical properties of the soil were as follows: Cd and Pb concentrations were 0.24 mg·kg^{-1} and 28.12 mg·kg^{-1}, pH was 6.88, organic substances content was 18.99 g·kg^{-1}, cation exchange capacity was 115.64 mmol·kg^{-1}, and the concentrations of available phosphorous, alkali-hydrolysable nitrogen, and available potassium were 41.35, 166.46 and 57.03 mg·kg^{-1}, respectively.

Experimental Design

Cd- and Pb-contaminated soil were simulated in a greenhouse pot experiment by air-drying the test soil (yellow earth), removing the weeds and gravel and then crushing and mixing it. The soil samples (2.5 kg each) were then placed in a plastic basin (diameters of 20 cm (top) and 16 cm (bottom), height of 18 cm) with a hole in the bottom in a tray. Cd and Pb (control group (CK)-0 mg·kg^{-1} Cd and 0 mg·kg^{-1} Pb; T1, 0.3 mg·kg^{-1} Cd and 250 mg·kg^{-1} Pb; T2, 1.5 mg·kg^{-1} Cd and 1250 mg·kg^{-1} Pb; T3, 3.0 mg·kg^{-1} Cd and 2500 mg·kg^{-1} Pb) were added exogenously onto the soil (an aqueous solution of CdCl$_2$·2.5H$_2$O and PbSO$_4$ were uniformly sprayed onto the soil, respectively), and base fertilizer containing (NH$_4$)$_2$SO$_4$ (843.6 mg·kg^{-1}), KH$_2$PO$_4$ (337.2 mg·kg^{-1}), and K$_2$SO$_4$ (885.7 mg·kg^{-1}) powders was applied. The treatment soil was thoroughly stirred

Table 1. Cultivars of tested hot peppers (*Capsicum annuum* L.).

No.	Cultivar	No.	Cultivar	No.	Cultivar
1	Huangpipaowang	5	Jinbaili	9	Guoxian2018
2	Zaozaerhao	6	Erjintiao	10	Guizuchaotianjiao
3	Wuhubopijiao	7	Luo28tiaojiao	11	Xianglaerjintiao
4	Qiangfeng7301	8	Youma	12	Erhaoniujiaojiao
13	Shuduhong	14	Zilameizi	15	Dahuangchaotianjiao

and maintained at a moisture content of 60-70% of the field moisture capacity and left to stand for 4 weeks. Subsequently, the hot pepper seedlings that had grown well and consistently were transplanted into the pots (180 pots in total) containing Cd- and Pb-contaminated soil (April 12, 2018). The experiment was conducted in a completely randomized block design with triplicate pots per treatment. All treatments were performed in the greenhouse at the Laboratory of Nuclear Waste and Environmental Security in Mianyang. The moisture content in the pots was regularly adjusted to approximately 60% of the field moisture capacity, and the rest of the environmental conditions were consistently maintained. After a 3-month growing period in the pots, the hot pepper cultivars were harvested (July 12, 2018) and the measurements were taken.

Measurement Methods

Biomass and Nitrate Content Measurement

The fresh fruits of hot pepper were first harvested and weighed. The roots and shoots (exclude the fruits) were carefully removed from the soils in pots, and washed with tap water, and carefully rinsed three times with deionized water. Then all plants were separated into roots, shoots, and fruits placed in paper bags. The paper bags were dried to a constant weight at 75ºC after 30 min at 105°C, and the roots, shoots, and fruits were weighed using an electronic balance again. The fresh hot pepper fruits were weighed to 0.5100 g and placed in a mortar. The samples were ground and poured into a 100 ml measuring flask with 30 ml deionized water. The samples were treated sequentially with 5 ml ammonia buffer (pH = 9.6), 30 ml deionized water, and powder-activated carbon of 0.5g with continuous agitation and then allowed to rest for 20 min. The nitrate content of hot pepper fruits was determined by the spectrometer (NanoDrop 2000, Beijing Purkinje General Instrument Co., Ltd, Beijing, China) at 219 nm.

Cd and Pb Concentration Measurement

The roots, shoots, and fruits were pulverized using a grinder and weighed accurately to 0.3000 g. Then, concentrated nitric acid (20 mL) was added, and

the samples were digested using a graphite furnace digester instrument (SH230N; Hanon Instruments, Jinan, China). After filtration, the Cd and Pb concentrations were determined using an ICP-OES (AA700; PerkinElmer, Waltham, Massachusetts, USA). $CdCl_2$ and $PbSO_4$ were used as standard materials for assurance control.

Translocation Factor (TF)

Translocation factor (TF) was used to evaluate the ability of hot pepper cultivars to translocate Cd (Pb) from roots to aboveground parts, and it was calculated using the following equation [26]:

$$\text{Translocation factor (TF)} = \frac{\text{the Cd (Pb) concentration of the aboveground parts (mg·kg}^{-1})}{\text{the Cd (Pb) concentration of the root (mg·kg}^{-1})}$$

Safety Standard and Statistical Analysis

The critical value of Cd and Pb in hot pepper cultivars at the safe production is 0.05 mg·kg^{-1} and 0.1 mg·kg^{-1} as recommended by WHO/FAO (Codex Alimentarius Commission. 2001), respectively. The Standards for nitrate content in fresh fruit vegetables is under 440 mg·kg^{-1} (GB 19338-2003). All treatments were replicated three times in the experiment, all data were analyzed with Excel 2013, SPSS 23.0 and Origin 9.0 for Windows. Values are expressed as mean±standard deviation (SD). Differences between treatments were considered significant at $p<0.05$.

Results and Discussion

Fruit Biomass

Cd and Pb are abundant and widespread toxic elements because they have adverse effects on plant growth, morphology, physiological and biochemical process [27]. Fruit biomass is a key factor to evaluate the tolerance of hot pepper cultivars in Cd- and Pb-contaminated soil. Fruit biomass (dry weight, DW) of the selected hot pepper cultivars under different Cd and Pb treatments was shown in Fig. 1. In the present study, the fruit biomass of 15 hot pepper cultivars under
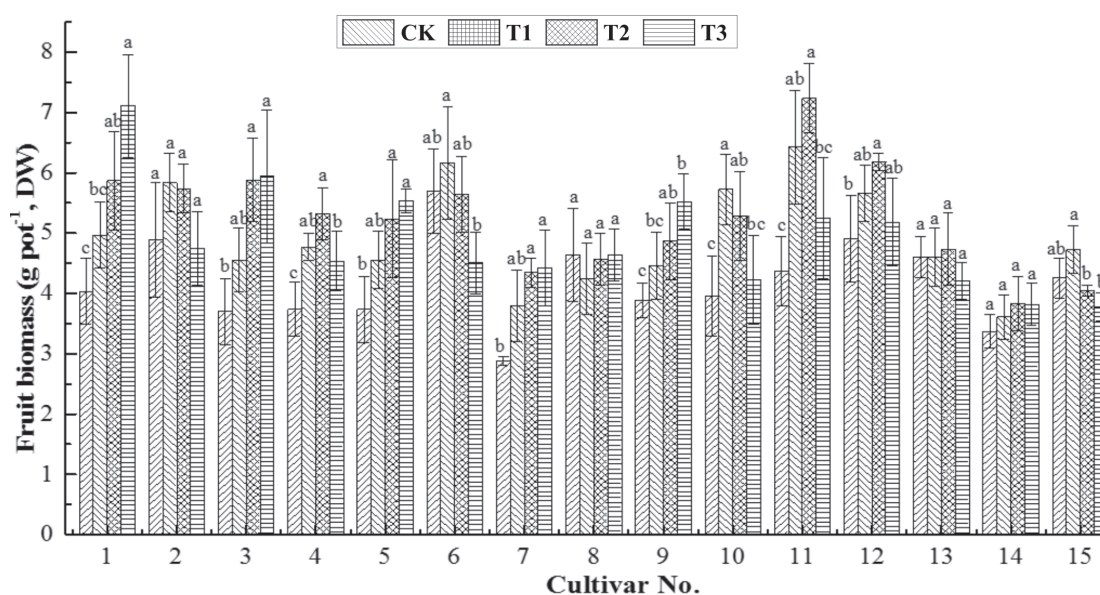
Fig. 1. Fruit biomass of hot pepper cultivars under four Cd and Pb treatments (control, T1, T2 and T3); lowercase letters indicate significant differences among different treatments in identical hot pepper fruit cultivars ($p<0.05$); total samples N = 180; replication n = 3).

three Cd and Pb treatments (T1, T2 and T3) did not decrease more significantly than the control groups, and the biomass of the six cultivars (Nos. 1, 3, 4, 5, 7 and 9) even significantly ($p<0.05$) increased in the T2 and T3 treatments (Fig. 1). These results indicated that hot pepper cultivars, especially the six cultivars (Nos. 1, 3, 4, 5, 7 and 9), have a higher tolerance to Cd and Pb contamination in agricultural soils compared with other crop cultivars, such as pakchoi (*Brassica chinensis* L.) [28], wheat (*Triticum aestivum* L.) [15] and tomato (*Solanum lycopersicum* L.) [10]. The stimulatory effect of heavy metals on plant growth may be a case of hormesis due to the physiological toxicity of heavy metals ion [29, 30]. This is another reason for promoting the growth of plants, and it is possible that the heavy metal ion serves as an enzyme activator for the metabolism of cytokinin [31, 32].

Nevertheless, the detailed mechanisms still need to be further researched. Furthermore, all hot pepper cultivars grew well and have not reduced yield in Cd and Pb agricultural contaminated soils, which may cause farmers to think that there is not Cd and Pb toxicity in hot pepper fruits, and pose a great risk to human health through the food chain. Therefore, the selection of hot pepper cultivars with low Cd and Pb accumulation to prevent heavy metals (Cd and Pb) entering the food chain is a feasible way for agricultural food safety.

Nitrate Content

Many study reports have shown that nitrate and nitrite will exert adverse effects on human health, and excess nitrate content may even cause cancer [33]. Most of the nitrates entering the human body is derived from

vegetables, thus we need to regulate the nitrate content in vegetables for decreasing the potential food safety risk [34].

The nitrate content of hot pepper fruits in three Cd and Pb treatments (T1, T2 and T3) is shown in Fig. 2, which indicates that the nitrate content of hot pepper fruits under two Cd and Pb treatments (T2 and T3) increased significantly ($p<0.05$) compared to the control (Fig. 2), and that the nitrate content in T3 treatments was higher than the T2 treatments. Under CK and T1, the nitrate content in fruits of all hot pepper cultivars were lower than 440mg/kg FW (GB 19338-2003). However, under T2 treatments, the nitrate content of Nos. 7, 10, 11, and 12 exceeded 440 mg·kg⁻¹ FW. The most nitrate content of hot pepper cultivars with T3 treatments were higher than 440 mg/kg FW, and only Nos. 2, 5 and 9 were lower than 440 mg/kg FW. These results showed that high-level Pb and Cd concentrations (T3 treatment) had a greater promotion to the nitrate content than the other treatments. Wei and Zhang [35] also found that the nitrate content in pakchoi increased significantly with the increase of heavy metals (Zn, Pb and Cd) concentration in soil. The possible reason is that the processes of plants absorption, translocation and utilizing nitrogen were changed in high heavy metal stress, which causes an increase of nitrate content [36]. In addition, the genotype is another reason that varied cultivars have different nitrate contents in heavy metals stress [37, 38].

Cd and Pb Concentrations in Hot Pepper Fruits

Significant ($p<0.05$) differences in fruit Cd (Pb) concentration were observed among 15 cultivars in
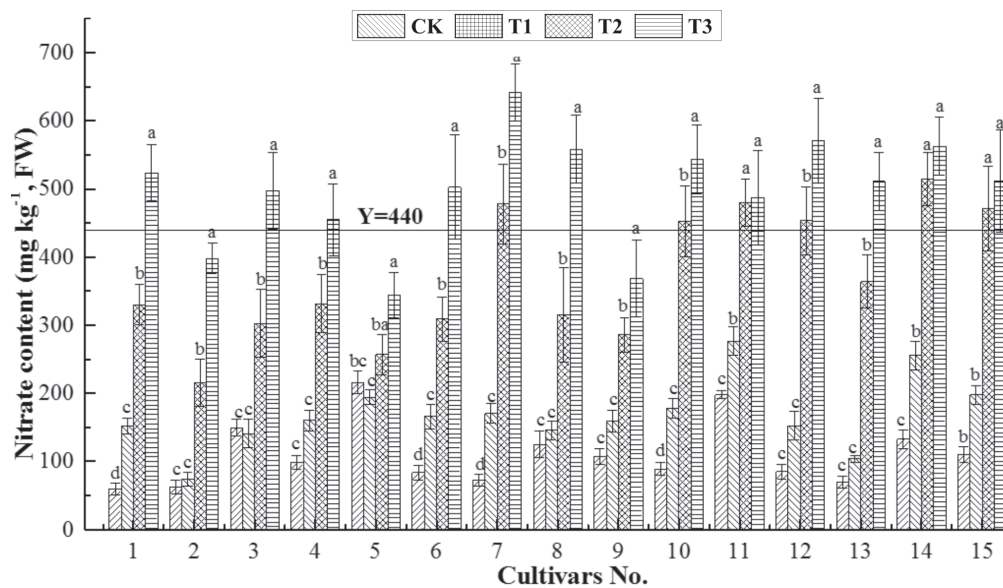
Fig. 2. Nitrate content (fresh weight, FW) of hot pepper fruits under Cd and Pb treatments (control, T1, T2 and T3); lowercase letters indicate significant differences among different treatments in identical hot pepper fruit cultivars ($p<0.05$; total samples N = 160; replication n = 3); Y line indicates the value of the standards for nitrate content.

three treatments (Table 2), ranging from 0.013 (0.007) to 0.041 (0.023), from 0.013 (0.021) to 0.058 (0.097), from 0.04 (0.075) to 0.11 (0.3), and from 0.42 (1.33) to 0.74 (3.39), with the mean of 0.024 (0.015), 0.036 (0.048), 0.077 (0.18) and 0.53 (2.28) mg/kg (FW) for control, T1-, T2-, and T3 treatments, respectively. In the same four treatments (control, T1, T2 and T3), the differences between the highest and lowest Cd concentrations in hot pepper fruits were 3.15-, 4.46-, 2.75- and 1.76-fold, respectively. Furthermore, the fruit Cd concentrations in the T3 treatments were, on average, 24.33 (16.35-36.8), 17.05 (9.31-33.1) and 7.28 (4.69-11.01) times higher than the control groups, T1 treatments and T2 treatments, respectively. In this study, the fruit Cd concentrations of all cultivars (FW) in the control groups were lower than the Codex General Standard (2001) (<0.05 mg·kg⁻¹, FW). Under the T1 and T2 treatments, 20% (3/15) and 80% (12/15) of hot pepper cultivars examined exceeded 0.05 mg/kg FW, respectively. However, all hot pepper cultivars examined in the T3 treatments exceeded 0.05 mg/kg FW. According to the Standard (2001), 3 hot pepper cultivars (Nos. 2, 8 and 11) were selected as low Cd accumulators, and the fruit Cd concentrations do not exceed the standard in the T1 and T2 treatments, which may engender a low risk to human health. Furthermore, Nos. 5 and 12 had higher Cd concentrations of hot pepper fruit compared with the other cultivars in the different treatments. By this way, 4 hot pepper cultivars (Nos. 1, 6, 8 and 11) met the standard of Pb concentration in hot pepper cultivar fruits (<0.1 mg·kg⁻¹, FW) in the T1 and T2 treatments, and the fruit Pb concentrations (FW) of all the cultivars exceeded 0.1 mg·kg⁻¹ in the T3 treatment (Table 2). Meanwhile, compared with the other cultivars, Nos. 3 and 12 had higher fruit Pb concentrations in the different treatments. In addition,

the Cd and Pb concentration of all hot pepper fruit cultivars exceed the Codex General Standard in T3 treatments (3.0 mg·kg⁻¹ Cd and 2500 mg·kg⁻¹). Similarly, Zhang et al. [11] reported that no low Cd and Pb lettuce (*Lactuca sativa* L.) cultivars were found under high Cd (1.428 mg·kg⁻¹) and Pb (498.660 mg·kg⁻¹) exposure. This might be because Cd and Pb concentration in fruits of hot pepper were determined by genotype difference and heavy metal (Cd and Pb) levels [21]. Therefore, the Cd and Pb concentrations in hot pepper fruits can be used to screen low Cd and Pb cultivars in certain Cd- and Pb-contaminated soils.

Cd and Pb Uptake, Distribution and Translocation

Cd and Pb Uptake and Distribution

The uptake and distribution of heavy metals have an important influence on the accumulation of heavy metals in edible plant parts, which have been reported for many crop cultivars [39, 40]. In the present study, heavy metals (iron) were absorbed by the plant roots, thus many researchers investigated the root uptake ability for clarifying the differences in heavy metals accumulation among cultivars [10, 18]. The Cd and Pb concentrations of roots, shoots (leaves and stems) and fruits (DW) of hot pepper cultivars in control, T1, T2 and T3 treatments are illustrated in Tables 3 and 4. The ratios of the shoot and fruit Cd concentrations for the five cultivars were 3.16-4.95, 4.12-8.34 and 8.56-12.52 in the control, T1 treatments and T2 treatments, respectively. The ratios of shoot and fruit Cd concentrations in the low Cd cultivars were significantly ($p<0.05$) higher than the high Cd cultivars, except in the T3 treatments.

Table 2. Concentrations of Cd and Pb (mg·kg⁻¹, fw) in fruits of hot pepper cultivars under four Cd and Pb treatments (control, T1, T2 and T3).

Cultivar No.		CK	T1	T2	T3
1	Cd	0.031±0.004bc	0.041±0.004b	0.092±0.008bc	0.51±0.037bcd
	Pb	0.013±0.002fg	0.043±0.006ef	0.094±0.004e	2.34±0.19def
2	Cd	0.014±0.002fg	0.013±0.002e	0.042±0.006fg	0.43±0.014d
	Pb	0.013±0.002fg	0.028±0.005g	0.21±0.021b	1.96±0.11fg
3	Cd	0.017±0.002fg	0.023±0.004cd	0.075±0.006d	0.50±0.056bcd
	Pb	0.023±0.004a	0.065±0.008b	0.24±0.045b	2.87±0.11bc
4	Cd	0.034±0.006b	0.052±0.007a	0.104±0.008ab	0.57±0.063b
	Pb	0.018±0.002bcd	0.04±0.002f	0.21±0.023bc	2.24±0.4ef
5	Cd	0.041±0.003a	0.058±0.005a	0.12±0.007a	0.54±0.062bc
	Pb	0.017±0.002bc	0.042±0.007f	0.23±0.021b	2.14±0.25efg
6	Cd	0.029±0.004bcd	0.036±0.004b	0.085±0.012cd	0.47±0.061bcd
	Pb	0.012±0.003g	0.043±0.004ef	0.092±0.005e	1.73±0.23gh
7	Cd	0.019±0.005ef	0.025±0.004c	0.058±0.005e	0.53±0.099bc
	Pb	0.014±0.002defg	0.056±0.006bcd	0.15±0.029d	2.73±0.47bcd
8	Cd	0.013±0.002g	0.017±0.002de	0.04±0.007g	0.42±0.022d
	Pb	0.007±0.001h	0.023±0.006g	0.075±0.009e	1.33±0.12h
9	Cd	0.026±0.003cd	0.042±0.006b	0.091±0.01bc	0.70±0.049a
	Pb	0.012±0.002g	0.048±0.007def	0.21±0.023bc	1.94±0.12fg
10	Cd	0.025±0.004d	0.035±0.006b	0.056±0.008ef	0.56±0.079b
	Pb	0.014±0.002efg	0.049±0.008def	0.23±0.027b	3.15±0.3ab
11	Cd	0.014±0.002fg	0.018±0.003cde	0.046±0.006efg	0.50±0.086bcd
	Pb	0.008±0.002h	0.021±0.003g	0.092±0.009e	1.93±0.19fg
12	Cd	0.034±0.004b	0.053±0.004a	0.11±0.013a	0.74±0.046a
	Pb	0.02±0.002ab	0.097±0.004a	0.3±0.038a	3.39±0.29a
13	Cd	0.019±0.003ef	0.037±0.004b	0.084±0.014cd	0.47±0.07bcd
	Pb	0.015±0.003cdef	0.048±0.008def	0.17±0.025cd	1.93±0.21fg
14	Cd	0.023±0.003de	0.051±0.008a	0.103±0.011ab	0.51±0.064bcd
	Pb	0.017±0.002bcde	0.053±0.009cde	0.21±0.025b	2.06±0.33fg
15	Cd	0.017±0.002fg	0.035±0.005b	0.076±0.01d	0.45±0.044cd
	Pb	0.018±0.003bc	0.06±0.008bc	0.22±0.03b	2.5±0.24cde

Note: 1. Data in the table is shown as mean±standard deviation (n = 3); 2. Different letters (a,b,c,d, e, etc.) refer to significant differences at the 0.05 level. (Total samples N = 160; Replication n = 3)

These results indicate that Cd translocation from shoots to fruits is more difficult in low Cd cultivars (Nos. 2, 8 and 11) compared to high Cd cultivars.

The root Cd concentration in No. 12 (high-Cd) was always the highest among the five cultivars, whereas it was only 20.23% and 18.14% higher than that in No. 2 (low-Cd) in T2 and T3 treatments, respectively (Table 3). The shoot Cd concentration in No. 12 (high-Cd) was also the highest among the five cultivars and was 184.65% and 207.55% higher than that in No. 2 (low-Cd) in T2 and T3 treatments, respectively (Table 3). Meanwhile, the variation of shoot and root Pb concentration between high-Pb cultivar and low-Pb cultivar was similar to the phenomenon of Cd (Table 4), and there were no significant correlations ($p < 0.05$) between the fruit Cd and Pb concentrations

Table 3. Concentrations of Cd (dry mass, DW) in roots, shoots (leaves and stems) and fruits of hot pepper cultivars under four Cd and Pb treatments (control, T1, T2 and T3),

Cultivar No.	Treatments	Roots Cd concentration	Shoots Cd concentration	Fruits Cd concentration
2-Low Cd Cultivar	Control	0.93±0.056e	0.35±0.047i	0.072±0.009c
	T1	1.19±0.15e	0.42±0.036i	0.07±0.005c
	T2	5.48±0.31d	2.15±0.20g	0.22±0.031b
	T3	13.01±1.91b	5.03±0.42de	2.31±0.12a
8-Low Cd Cultivar	Control	0.87±0.026e	0.32±0.035i	0.073±0.011b
	T1	1.48±0.06e	0.57±0.085hi	0.068±0.01b
	T2	6.04±0.22cd	2.63±0.50fg	0.21±0.03b
	T3	12.73±1.53b	5.08±0.18de	2.62±0.24a
11-Low Cd Cultivar	Control	0.82±0.042e	0.45±0.078hi	0.091±0.008c
	T1	1.32±0.085e	0.56±0.03hi	0.12±0.023c
	T2	7.47±0.47c	3.34±0.50f	0.39±0.029b
	T3	14.8±1.51a	7.31±0.69c	2.51±0.14a
5-High Cd Cultivar	Control	0.96±0.082e	0.57±0.09hi	0.17±0.023c
	T1	1.61±0.12e	1.03±0.10hi	0.25±0.031bc
	T2	6.48±0.39cd	4.77±0.54e	0.54±0.053b
	T3	14.52±2.06a	9.92±1.07b	2.67±0.40a
12-High Cd Cultivar	Control	1.03±0.08e	0.67±0.12hi	0.19±0.021c
	T1	1.58±0.16e	1.53±0.14gh	0.28±0.017c
	T2	6.59±0.64cd	6.12±0.51d	0.58±0.085b
	T3	15.37±1.58a	15.47±2.42a	3.82±0.23a

Note: 1. Data in the table is shown as mean ± standard deviation (n = 3); 2. Different letters (a,b,c,d, e, etc.) refer to significant differences at the 0.05 level. (Total samples N = 160; Replication n = 3)

and the root Cd and Pb concentrations (Table 5). Therefore, the possible reason for the higher fruit Cd and Pb concentrations in high Cd and Pb cultivars is that the high (Cd and Pb) cultivars increased the ability to absorb Cd and Pb through its roots and shoots, especially the shoots being more important, which were influenced the plant genotype, the bioavailability of heavy metals, and plant metabolic pattern [15, 41]. Although Xue et al. [42] also found that low-Cd pakchoi (*Brassica chinensis* L.) cultivar 'AJKSHY' and 'BMG' had a higher Cd concentration in the roots than the high Cd cultivar 'SYM' and 'AJSZQ', no significant difference in root Cd concentrations was found among the four cultivars. Similarly, no significant differences of root Cd concentrations in low Cd rice cultivar 'Sasanishiki' and the high Cd cultivar 'Habataki' were also reported by Uraguchi et al. [43]. These results indicated that the Cd and Pb uptake in roots is not a major factor determining the significant difference in fruit Cd and Pb concentrations between high and low Cd and Pb hot pepper cultivars. Therefore, the Cd and Pb concentrations in hot pepper fruits is less correlation

with root Cd and Pb concentrations compared to the shoot Cd and Pb concentrations (Table 5).

Cd and Pb Translocation

The translocation factor is calculated as the ratio of the Cd and Pb concentrations in plant aboveground parts to the Cd and Pb concentrations in plant roots, which indicated the capacity of plants to translocate Cd and Pb from roots to aboveground parts [44]. In this study, low Cd and Pb cultivars always had a lower translocation factor than the two high Cd and Cd cultivars (Fig. 3). This result shows that low Cd and Pb cultivars may have a greater ability to detain Cd in roots compared with high Cd and Pb cultivars. Meanwhile, the root Cd and Pb concentrations in the high Cd and Pb cultivars were not significant differences ($p<0.05$) compared to the low Cd and Pb cultivars; however, greater Cd and Pb translocation factors were observed in high Cd and Pb cultivars, respectively (Fig. 3). In addition, significant positive relationships ($p<0.05$) were also found between fruit Cd and Pb concentrations

Table 4. Concentrations of Pb (dry mass, DW) in roots, shoots (leaves and stems) and fruits of pepper cultivars under four Cd and Pb treatments (control, T1, T2 and T3).

Cultivar No.	Treatments	Roots Pb concentration	Shoots Pb concentration	Fruits Pb concentration
1-Low Pb Cultivar	Control	6.72±1.15g	1.14±0.11h	0.073±0.009h
	T1	68.95±9.30f	34.58±5.84fg	0.24±0.032h
	T2	216.18±13.18de	130.56±11.76cd	0.54±0.031gh
	T3	303.64±25.23b	132.96±9.07cd	11.1±1.62c
6-Low Pb Cultivar	Control	5.77±0.68g	0.93±0.071h	0.067±0.015h
	T1	67.52±6.54f	36.25±2.01fg	0.25±0.023h
	T2	206.18±22.33de	120.67±15.34cde	0.53±0.027gh
	T3	315.06±26.82b	147.16±4.14c	9.93±1.29d
8-Low Pb Cultivar	Control	4.77±0.54g	0.64±0.056h	0.042±0.006h
	T1	65.79±7.02f	30.55±2.78gh	0.13±0.017h
	T2	230.2±19.55d	122.69±9.96cde	0.44±0.051gh
	T3	305.26±16.05b	118.99±15.19cde	7.67±0.68e
11-Low Pb Cultivar	Control	5.44±0.88g	0.77±0.10h	0.046±0.01h
	T1	69.52±7.30f	30.53±1.33gh	0.12±0.02h
	T2	199.23±11.11de	98.08±7.46e	0.53±0.049gh
	T3	321.19±33.60b	104.88±15.98de	9.83±1.4d
3-High Pb Cultivar	Control	6.59±0.76g	1.49±0.19h	0.13±0.021h
	T1	72.76±5.96f	53.52±5.45fg	0.38±0.047gh
	T2	226.89±24.31de	293.87±32.2b	1.4±0.26fg
	T3	332.17±22.81b	349.25±43.49a	16.49±0.63b
12-High Pb Cultivar	Control	7.17±0.78g	1.89±0.17h	0.12±0.02h
	T1	80.43±7.02f	65.10±6.51f	0.56±0.017gh
	T2	261.56±28.63c	365.30±59.29a	1.76±0.2f
	T3	367.77±45.24a	368.12±26.86a	19.51±1.67a

Note: 1. Data in the table is shown as mean ± standard deviation (n = 3); 2. Different letters (a,b,c,d, e, etc.) refer to significant differences at the 0.05 level. (Total samples N = 160; Replication n = 3)

to Cd and Pb translocation factors (Table 5), suggesting that translocation ability is an important factor that can affect Cd and Pb accumulation in fruits. This may be attributed to the fact that movement of Cd and Pb into fruits probably occurs via phloem, which is a process of retranslocation from shoots (stems and leaves) to fruits [40]. Liu et al. [13] investigated the translocation factor of low- and high-Cd Chinese cabbage (*Brassica pekinensis (Lour.) Rupr.*) cultivars, and found that the translocation factor was highly correlated with the concentration in edible plant parts of Chinese cabbage cultivars. In the present study, significant (*p*<0.05)

Table. 5. Relationships, given as r values (n=15(Cd) and 18(Pb)), between hot pepper fruit Cd and Pb concentrations and the Cd and Pb concentrations in shoots and roots, and Cd and Pb translocation factors under different treatments (significant at *P*<0.05).

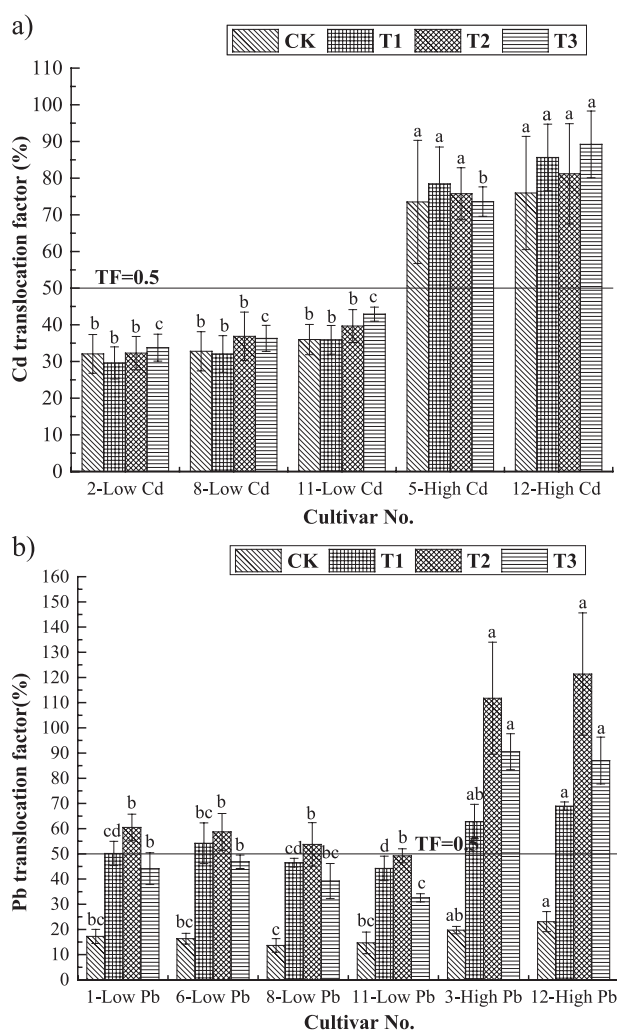| Treatments	Root concentration		Shoot concentration		Translocation factor	
	Cd	Pb	Cd	Pb	Cd	Pb
T1	0.706	0.585	0.916*	0.915*	0.934*	0.837*
T2	0.428	0.633	0.935*	0.920*	0.879*	0.823*
T3	0.287	0.625	0.808*	0.939*	0.764*	0.895*

a)

b)

Fig. 3. Difference of translocation factor in low- Cd (a) and -Pb (b) and high-Cd (a) and -Pb (b) hot pepper cultivars under four Cd and Pb treatments (control, T1, T2 and T3); lowercase letters indicate significant differences among different cultivars in identical Cd and Pb treatments ($p < 0.05$; total samples N = 160; replication n = 3); Y line indicates the value of translocation factor (Y = 0.5).

positive correlation was also found between the Cd and Pb concentrations in fruits and in shoots (Table 5). Nevertheless, the determination of whether the stem or leaf provides a bigger contribution to Cd and Pb concentration in fruits of hot pepper still needs more experimentation.

Conclusions

The results of the present study indicate that the ability to accumulate Cd and Pb in fruits are significant differences among the hot pepper cultivars ($p < 0.5$). Hot pepper cultivars Nos. 8 (Youma) and 11 (Xianglaerjintiao) were selected as low Cd and Pb cultivars in this study. Nevertheless, the nitrate content of No. 11 (Xianglaerjintiao) exceeded 440 mg·kg^{-1} FW, thus No. 11 (Xianglaerjintiao) can be grown to

increase food safety compare to No. 8 (Youma). The differences in fruit Cd and Pb concentrations between low and high cultivars were owing to variations of Cd and Pb translocation factors and shoot Cd and Pb concentrations rather than to the roots Cd concentration. In addition, further experiments should be conducted to demonstrate whether the accumulation of heavy metal (Cd and Pb) in hot pepper fruits mainly originates from phloem transport or xylem transport, which is conducive to breeding for low Cd and Pb cultivars.

Acknowledgements

Conflict of Interest

The authors declare that they have no competing interests.

References

1. SHAHEEN N., IRFAN N.M., KHAN I.N., ISLAM S., ISLAM M.S., AHMED M.K. Presence of heavy metals in fruits and vegetables: Health risk implications in Bangladesh. Chemosphere, **152**, 431, **2016**.
2. ANTONIADIS V., SHAHEEN S.M., LEVIZOU E., SHAHID M., NIAZI N.K., VITHANAGE M., OK Y.S., BOLAN, N., RINKLEBE J. A critical prospective analysis of the potential toxicity of trace element regulation limits in soils worldwide: Are they protective concerning health risk assessment? - A review. Environment International, **127**, 819, **2019**.
3. LU L., LI J., HU A., MULLA S., YANG J., YU C.P. Microbial community structure analysis and isolation of vanadium-resistant strains in vanadium mining-impacted soil. Journal of Soil and Water Conservation, **74** (3), 296, **2019**.
4. ZHAO F.J., MA Y., ZHU Y.G., TANG Z., MCGRATH S.P. Soil Contamination in China: Current Status and Mitigation Strategies. Environmental Science & Technology, **49**(2), 750, **2015**.
5. ZHANG H., GUO Q., YANG J., MA J., CHEN G., CHEN T., ZHU G., WANG J., ZHANG G., WANG X., SHAO C. Comparison of chelates for enhancing *Ricinus communis* L. phytoremediation of Cd and Pb contaminated soil. Ecotoxicology and Environmental Safety, **133**, 57, **2016**.
6. BOPARAI H.K., JOSEPH M., O'CARROLL D.M. Kinetics and thermodynamics of cadmium ion removal by adsorption noto nano zerovalent iron particles. Journal of Hazardous Materials, **186** (1), 458, **2011**.
7. FAHMI A.H., SAMSURI A.W., JOL H., SINGH D. Bioavailability and leaching of Cd and Pb from contaminated soil amended with different sizes of biochar. Royal Society Open Science, **5** (11), 181328, **2018**.
8. WANG L., HUANG L., XIA H., LI H., LI X., LIU X. Application of a multi-electrode system with polyaniline auxiliary electrodes for electrokinetic remediation of

chromium-contaminated soil. Separation and Purification Technology, **224**, 106, **2019**.

9. LIU W., ZHOU Q., AN J., SUN Y., LIU R. Variations in cadmium accumulation among Chinese cabbage cultivars and screening for Cd-safe cultivars. Journal of Hazardous Materials, **173** (1-3), 737, **2010**.

10. XU Z. M., TAN X.Q., MEI X.Q., LI Q.S., ZHOU C., WANG L.L.,YE H.J., YANG P. Low-Cd tomato cultivars (*Solanum lycopersicum* L.) screened in non-saline soils also accumulated low Cd, Zn, and Cu in heavy metal-polluted saline soils. Environmental Science and Pollution Research, **25** (27) 27439, **2018**.

11. ZHANG K., YUAN J., KONG W., YANG Z. Genotype variations in cadmium and lead accumulations of leafy lettuce (*Lactuca sativa* L.) and screening for pollution-safe cultivars for food safety. Environmental Science: Processes & Impacts, **15** (6), 1245, **2013**.

12. LI X., ZHOU Q., WEI S., REN W. Identification of cadmium-excluding welsh onion (*Allium fistulosum* L.) cultivars and their mechanisms of low cadmium accumulation. Environmental Science and Pollution Research, **19** (5), 1773, **2012**.

13. LIU W., ZHOU Q., SUN Y., LIU R. Identification of Chinese cabbage genotypes with low cadmium accumulation for food safety. Environmental Pollution, **157** (6), 1961, **2009**.

14. ZHANG Z., JIN F., WANG C., LUO J., LIN H., XIANG K., LIU L., ZHAO M., ZHANG Y., DING H., ZHOU S., SHEN Y., PAN, G. Difference between Pb and Cd Accumulation in 19 Elite Maize Inbred Lines and Application Prospects. Journal of Biomedicine and Biotechnology, **2012**, 1, **2012**.

15. LIU W., LIANG L., ZHANG X., ZHOU Q. Cultivar variations in cadmium and lead accumulation and distribution among 30 wheat (*Triticum aestivum* L.) cultivars. Environmental Science and Pollution Research, **22** (11), 8432, **2015**.

16. WU Z., WU W., ZHOU S., WU S. Mycorrhizal Inoculation Affects Pb and Cd Accumulation and Translocation in Pakchoi (*Brassica chinensis* L.). Pedosphere, **26** (1), 13, **2016**.

17. ZHOU Y., XUE M., YANG Z., GONG Y., YUAN J., ZHOU C., HUANG B. High cadmium pollution risk on vegetable amaranth and a selection for pollution-safe cultivars to lower the risk. Frontiers of Environmental Science & Engineering, **7** (2), 219, **2013**.

18. LIU B.Q., WU F.F., DAI X.Z., LIU F., MI B.L. Variations of Cadmium Accumulation and Translocation in Different Pakchoi Cultivars and Screening for Cd-Pollution-Safe Cultivars Using Cluster Analysis. Polish Journal of Environmental Studies, **28** (4), 2215, **2019**.

19. SU Y., WANG X., LIU C., SHI G. Variation in cadmium accumulation and translocation among peanut cultivars as affected by iron deficiency. Plant and Soil, **363** (1-2), 201, **2013**.

20. HAGHIGHI M., KAFI M., PESSARAKLI M., SHEIBANIRAD A., SHARIFINIA M.R. Using kale (*Brassica oleraceavar. acephala*) as a phytoremediation plant species for lead (Pb) and cadmium (Cd) removal in saline soils. Journal of Plant Nutrition, **39** (10), 1460, **2016**.

21. TANG L., HAMID Y., ZEHRA A., SAHITO Z.A., HE Z., HUSSAIN B., GURAJALA H.K., YANG X. Characterization of fava bean (*Vicia faba* L.) genotypes for phytoremediation of cadmium and lead co-contaminated soils coupled with agro-production. Ecotoxicology and Environmental Safety, **171**, 190, **2019**.

22. HORD N.G., TANG Y., BRYAN N.S. Food sources of nitrates and nitrites: the physiologic context for potential health benefits. The American Journal of Clinical Nutrition, **90** (1), 1, **2009**.

23. CORRADINI F., CORREA A., MOYANO M.S., SEPÚLVEDA P., QUIROZ C. Nitrate, arsenic, cadmium, and lead concentrations in leafy vegetables: expected average values for productive regions of Chile. Archives of Agronomy and Soil Science, **64** (3), 299, **2017**.

24. DELLAVALLE C.T., XIAO Q., YANG G., SHU X.O., ASCHEBROOK-KILFOY B., ZHENG W., LI H., JI B.T., ROTHMAN N., CHOW W.H., GAO Y.T., WARD M.H. Dietary nitrate and nitrite intake and risk of colorectal cancer in the Shanghai Women's Health Study. International Journal of Cancer, **134** (12), 2917, **2014**.

25. ANTONIOUS G.F. Distribution of seven heavy metals among hot pepper plant parts. Journal of Environmental Science and Health Part b, **51** (5), 309, **2016**.

26. CHEN L., LONG C., WANG D., YANG J. Phytoremediation of cadmium (Cd) and uranium (U) contaminated soils by *Brassica juncea* L. enhanced with exogenous application of plant growth regulators. Chemosphere, **242**, 125112, **2020**.

27. JOHN R., AHMAD P., GADGIL K., SHARMA S. Cadmium and lead-induced changes in lipid peroxidation, antioxidative enzymes and metal accumulation in Brassica juncea L. at three different growth stages. Archives of Agronomy and Soil Science, **55** (4), 395, **2009**.

28. CHEN Y., LI T., HAN X., DING Z., YAN X., JIN Y. Cadmium accumulation in different pakchoi cultivars and screening for pollution-safe cultivars. Journal of Zhejiang University-SCIENCE B, **13** (7), 494, **2012**.

29. PILON-SMITS E.A., QUINN C.F., TAPKEN W., MALAGOLI M., SCHIAVON M. Physiological functions of beneficial elements. Current Opinion in Plant Biology, **12** (3), 267, **2009**.

30. POSCHENRIEDER C., CABOT C., MARTOS S., GALLEGO B., BARCELÓ J. Do toxic ions induce hormesis in plants? Plant Science, **212**, 15, **2013**.

31. XIN J., HUANG B., LIU A., ZHOU W., LIAO K. Identification of hot pepper cultivars containing low Cd levels after growing on contaminated soil: uptake and redistribution to the edible plant parts. Plant and Soil, **373** (1-2), 415, **2013**.

32. NYITRAI P., BÓKA K., GÁSPÁR L., SÁRVÁRI É., KERESZTES Á. Rejuvenation of Ageing Bean Leaves under the Effect of Low-Dose Stressors. Plant Biology, **6** (6), 708, **2004**.

33. IAMMARINO M., DI TARANTO A., CRISTINO M. Monitoring of nitrites and nitrates levels in leafy vegetables (spinach and lettuce): a contribution to risk assessment. Journal of the Science of Food and Agriculture, **94** (4), 773, **2014**.

34. LI S., LI J., ZHANG B., LI D., LI G., LI Y. Effect of different organic fertilizers application on growth and environmental risk of nitrate under a vegetable field. Scientific Reports, **7**, 17020, **2017**.

35. WEI C.X., ZHANG X. Effects of Zinc lead and cadmium contamination on nitrate contents in pakchoi. Journal of Southwest University, **27** (4), 436, **2005**.

36. DEMIRAL M.A., AY M., SORAL F., TEKIN M. Effect of nitrogen on growth and nitrate accumulation of some leafy vegetables. Journal of Adnan Menderes University, Agricultural Faculty, **6** (2), 3, **2009**.

37. CAO F., WANG R., CHENG W., ZENG F., AHMED I.M., HU X., ZHANG G., WU F. Genotypic and environmental

variation in cadmium, chromium, lead and copper in rice and approaches for reducing the accumulation. Science of The Total Environment, **496**, 275, **2014**.

38. WANG X., BAI J., WANG J., LE S., WANG M., ZHAO Y. Variations in cadmium accumulation and distribution among different oilseed rape cultivars in Chengdu Plain in China. Environmental Science and Pollution Research, **26** (4), 3415, **2019**.

39. DING C., ZHANG T., WANG X., ZHOU F., YANG Y., YIN Y. Effects of soil type and genotype on lead concentration in rootstalk vegetables and the selection of cultivars for food safety. Journal of Environmental Management, **122**, 8, **2013**.

40. HUANG B., XIN J., DAI H., ZHOU W., PENG L. Identification of low-Cd cultivars of sweet potato (*Ipomoea batatas* (L.) Lam.) after growing on Cd-contaminated soil: uptake and partitioning to the edible roots. Environmental Science and Pollution Research, **22** (15), 11813, **2015**.

41. ZHU Y., YU H., WANG J., FANG W., YUAN J., YANG Z. Heavy Metal Accumulations of 24 Asparagus Bean Cultivars Grown in Soil Contaminated with Cd Alone and with Multiple Metals (Cd, Pb, and Zn). Journal of Agricultural and Food Chemistry, **55** (3), 1045, **2017**.

42. XUE M., ZHOU Y., YANG Z., LIN B., YUAN J., WU S. Comparisons in subcellular and biochemical behaviors of cadmium between low-Cd and high-Cd accumulation cultivars of pakchoi (*Brassica chinensis* L.). Frontiers of Environmental Science & Engineering, **8** (2), 226, **2014**.

43. URAGUCHI S., MORI S., KURAMATA M., KAWASAKI A., ARAO T., ISHIKAWA S. Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. Journal of Experimental Botany, **60** (9), 2677, **2009**.

44. AFONSO T.F., DEMARCO C.F., PIENIZ S., CAMARGO F.A.O., QUADRO M.S., ANDREAZZA R. Potential of Solanum viarum Dunal in use for phytoremediation of heavy metals to mining areas, southern Brazil. Environmental Science and Pollution Research, **26** (23), 24132, **2019**.