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Original Research

Variations of Cadmium Accumulation and Translocation in Different Pakchoi Cultivars and Screening for Cd-Pollution-Safe Cultivars Using Cluster Analysis

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Abstract

Screening and breeding of pollution-safe cultivars (PSCs) has become one of the most direct and cost-effective methods for reducing the health risks of heavy metal-contaminated soil. In this paper we identified and screened out cadmium (Cd)-pollution-safe pakchoi cultivars from 30 pakchoi genetype materials through preliminary screening experiments and re-screening experiments in field conditions. We found that in preliminary screening experiments, the Cd uptake displayed significant variability among the 30 selected pakchoi cultivaters grown in soil with a Cd concentration of 0.51 mg/kg. 11 out of the 30 pakchoi cultivars belonged to the low-Cd accumulated cluster classified by cluster analysis, which had the potential to be Cd-PSCs. The re-screening experiments under different Cd levels of contaminated soils in combination with the studies of the enrichment factors (EFs) and translocation factors (TFs) further confirmed the consistency and genotypic stability of the low-Cd accumulating traits of the potential Cd-PSCs. This study proves the feasibility of applying cluster analysis in the process of identifying PSCs.

Keywords: cadmium, pollution-safe cultivar, pakchoi, cluster analysis

Introduction

Soil contamination by heavy metals has become an increasingly serious environmental problem throughout the world as a consequence of various anthropogenic activities such as industrial production and freewill voidance of wastes [1-3]. The pollution has a negative effect on the eco-environment as well as agricultural production, and eventually affects human and animal health [4, 5]. Although the remediation of polluted soil with heavy metals is a difficult, time-consuming, and costly operation, in recent years many

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Table 1. Selected pakchoi genotypes.

Cultivar name	No.	Cultivar name	No.	Cultivar name	No.
Huangjin	1	Baiyu 2	11	Tuzitui	21
Jiahui	2	Dongjing 1	12	Shanghaiqing	22
Aikangjiaxue	3	Lvxiu	13	Jingpin	23
Chunlaizaobai	4	Huojian	14	Biangeng	24
Xiaobaicai 536	5	Jinsha	15	Lusan	25
Xianfeng	6	Chunlaijiabai	16	Degaoxialv	26
Danping	7	Baiyu 1	17	Nanhu 161	27
Cuimei	8	Kangrejiangjun	18	Suzhouqin	28
Bailingliren	9	Lvbao	19	Xialv	29
Jinghua 1	10	Baixuegongzhu	20	Chunbulao	30

efforts have been devoted to heavy metal-polluted soil remediation by in situ remediation methods such as stabilization [6-8] and phytoremediation [9-11]. Among them, phytoremediation technology is considered an environmentally friendly treatment that is receiving increasing attention domestically and overseas because of its low cost, in situ, and other advantages [12, 13]. That is to screen for hyper-accumulated plants which can tolerate and extract high levels of heavy metals from soil and store them in harvestable shoot tissue. However, many of the hyper-accumulated plants are slow-growing, and given the large areas of polluted soil, the cycle of phytoremediation is too long to quickly decontaminate many polluted soils, severely constraining their potential to be used in soil restoration.

Cadmium (Cd) has been considered one of the most deleterious heavy metals, and it is easy to be accumulated by many crops that are detrimental to human health through the soil biology chain and ranked 7th out of the top 20 toxins [14]. Cereals and vegetables are the main crops and more than 80% of Cd entering the human body comes from them [15]. Pokchoi (Brassica campestris ssp.Chinensis Makino) is a kind of cruciferous leafy vegetable that is cultivated widely across China, which easily causes the risks of human exposure to Cd through consumption of pokchoi [16]. Therefore, it is imperative to find an instantaneous and effective method to reduce the potential health risks of Cd entering the human food chain through the consumption of pokchoi. Genetic differences in Cd uptake and translocation in food crops have been observed by many published papers, and the variation existed not only among cultivar species and even within the genetypes but also in cultivar parts [17-19]. Selecting appropriate cultivars with a high tolerance to Cd-contaminated soil and low Cd accumulation in the edible parts are reasonable to minimize soil-to-crops of Cd and reduce the risks of Cd to human and animal health. Based on these, Yu et al. proposed the concept of pollution-safe cultivars (PSCs), i.e. cultivars in which edible parts accumulate specific pollutants at a level low enough for safe consumption even when grown in contaminated soil [20]. Recently, selecting PSCs grown in heavy metal-contaminated soil was considered one of the feasible and efficient methods for minimizing the influx of heavy metals to the human body and reducing the risks of human exposure to heavy metals through crop consumption [21-23]. Indeed, a Cd-pollution-safe wheat cultivar named "Strongfield" has been commercially developed and introduced in Canada in the past few years [24].

The present study was undertaken to investigate genetic differences in Cd uptake and translocation in pokchoi cultivars and selecting PSCs. Considering that environmental factors can significantly affect the availability of heavy metals and the content of heavy metals in cultivars [25-27], identifying and screening pollution-safe pakchoi cultivars was conducted in field-culture experiments, and Cd-pollution-safe pakchoi cultivars were screened out from 30 pakchoi genetype materials. Specifically, cluster analysis was applied in the screening experiments.

Materials and Methods

Pakchoi Materials

30 pakchoi genotypes were collected from the Mawangdui Seed Market in Changsha, China, and the names and numbers of the selected 30 cultivars are listed in Table 1.

Screening Experiments

Preliminary screening experiments were conducted by covering plants with voile in a demonstration garden of the Research Institute of Vegetables, Hunan Academy of Agricultural Sciences, in Changsha, Hunan Province, China. Soil pH, organic matter, total N, total

Table 2. Physicochemical characteristics of tested soils.

Total Cd/ mg/kg	Soil pH	Organic matter/g/kg	Total N/g/kg	Total P/g/kg	Available K/ mg/kg
0.30	5.57	24.91	1.43	0.98	140.36
0.39	5.30	22.42	1.03	1.12	119.69
0.64	5.82	25.94	1.06	1.26	168.88
1.46	5.18	21.08	1.33	1.25	155.39
2.12	5.24	24.30	1.07	1.04	187.14

P, available K, and total Cd were 5.36, 22.60 g/kg, 1.29 g/kg, 0.49 g/kg, 130.20 mg/kg, and 0.51 mg/kg, respectively, and the Cd concentration of selected soil was slightly beyond the limited value of Cd (0.3 mg/kg), referring to the Farmland Environmental Quality Evaluation Standard for Edible Agricultural Products (HJ 332-2006). Seeds were sowed directly into the selected soil in September 2014, and the mature plants were randomly collected in January 2015. The collected samples were sequentially washed, weighted, and analyzed for Cd concentrations.

Re-screening experiments were conducted followed by preliminary screening experiments in Xiangtan, Hunan Province, China, where soil suffered different Cd-level contamination due to the intrusion of wastewater, particle powder, and mine gangue. According to the field survey data on Cd concentrations in vegetable lands in China [28] and the published literature by Wang et al. [29], Chinese vegetable lands fall into three categories: low Cd-contaminated soil with a 0.30-0.60 mg/kg Cd concentration, moderate Cd-contaminated soil a 0.60-1.00 mg/kg Cd concentration, and high Cd-contaminated soil with a concentration higher than 1.00 mg/kg. Thus, the experiments were conducted on five typical areas with different Cd contamination levels ranging from 0.30 to 2.12 mg/kg. The physicochemical characteristics of the five soils are shown in Table 2. The treatment of the seeds and mature plants was the same as described in preliminary screening experiments.

Data Analysis

Two indicators were used to measure the safety of edible parts of pakchoi grown in Cd-contaminated soils: the maximum permissible concentration of Cd of 0.20 mg/kg in leaf vegetables for safe consumption (GB2762-2012), and the maximum concentration of 0.05 mg/kg for "no-polluted vegetables" (GB18406.1-2001). Enrichment factor (EF) was used to evaluate the ability of pakchoi to accumulate Cd, while translocation factor (TF) was related to the capacity of pakchoi cultivars to translocate Cd from roots to edible parts [30, 31]. Combining the analysis of Cd uptakes in edible parts of pakchoi under different Cd concentrations in soil with EFs and TFs were used for understanding the relationship between the soil contamination level and pakchoi species. The values of EFs and TFs were considered as a screening standard (EF<1.0 and TF<1.0), which can be calculated based on the following equations:

$$EF = C_1/C_0$$

$$TF = C_1/C_2$$

…where C_0 is the Cd concentration in the soil, C_1 is the Cd concentration of the edible part in pakchoi cultivar, and C_2 is the Cd concentration of the root in pakchoi cultivar.

Data were analyzed by Origin 8.5 and Excel 2016, and results of cluster analysis were obtained by DPS 2.0 (data processing system 2.0).

Results and Discussions

From Fig. 1, the differences among the Cd accumulation in 30 pakchoi cultivars were significant. The selected pakchoi cultivars were classified by cluster analysis, and the results are shown in Fig. 2. It was obvious that the selected pakchoi cultivars fall into four clusters at classification distance of about 0.07, and for the convenience of screening, the first and second

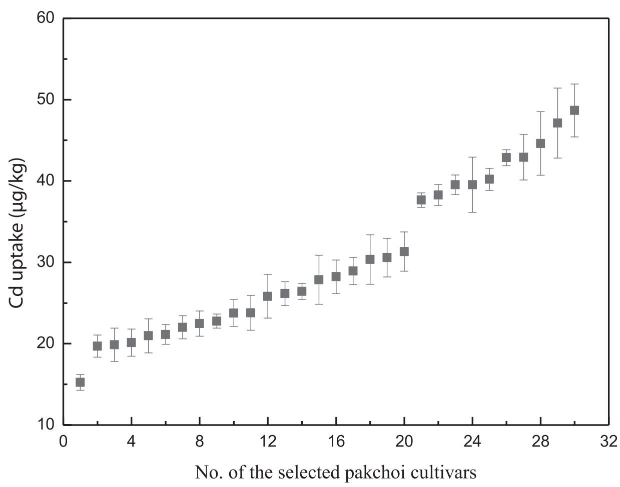

Fig. 1. Cd uptakes in edible parts of pakchoi cultivars under Cd concentration of 0.51 mg/kg.

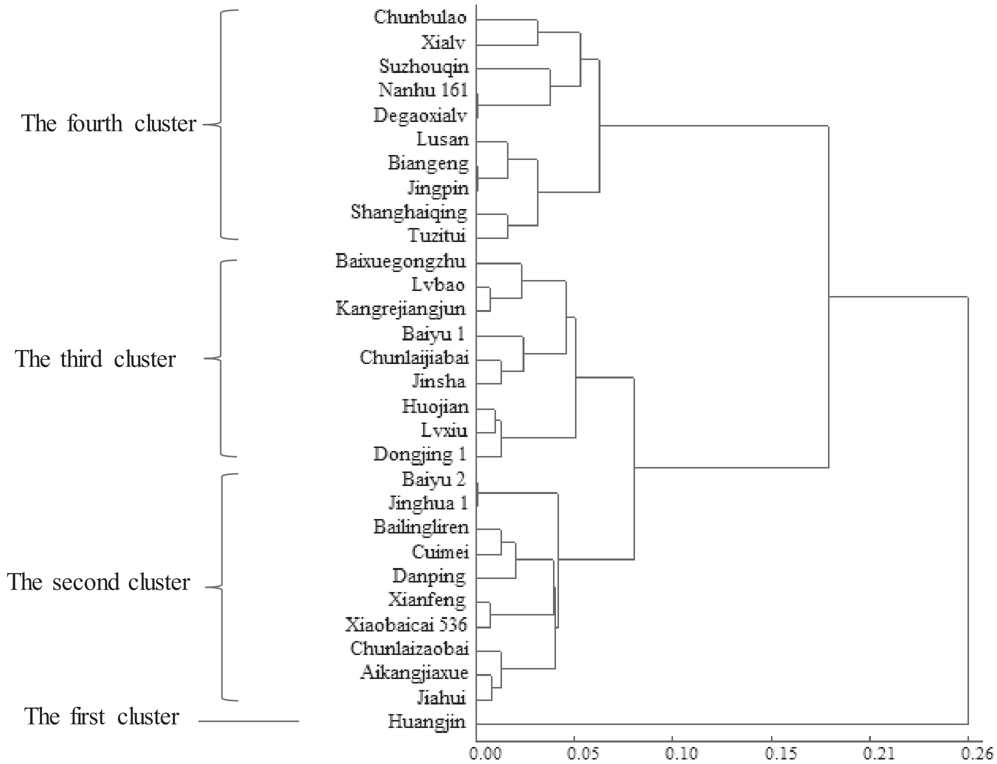

Fig. 2. Results of cluster analysis.

clusters in Fig. 2 were put into one cluster renamed as first cluster (low-Cd accumulated cluster), and the other two clusters were accordingly renamed as second cluster (medium-Cd accumulated cluster) and third cluster (high-Cd accumulated cluster).

Table 3 lists the Cd uptakes of ten representative pakchoi cultivaters of the three clusters based on the results of cluster analysis. Among them, Huangjin, Aikangjiaxue, Xiaobaicai 536, and Bailingliren belonged to the low-Cd accumulated cluster; Baixuegongzhu and Lvxiu belonged to the medium-Cd accumulated cluster; and the rest belonged to the high-Cd accumulated cluster. The average value of Cd uptakes in the third cluster was 2.07-fold higher than that in first cluster, implying that pakchoi cultivars in the first cluster were much more likely to be the Cd-PSCs than in the other two clusters.

Moreover, undoubtedly the Cd concentration in cultivars is also highly contingent on the Cd concentration in soil, and the Cd uptake values varied in different Cd-contaminated soils [32-34]. Therefore, the Cd-contaminated level in soil should also be taken into consideration for screening for Cd-polluted-safe pakchoi cultivars. In this paper, to clear the contribution

Table 3. Cd uptakes in edible parts of ten selected representative pakchoi cultivars under Cd concentration of 0.51 mg/kg.

Cd accumulated type	Cultivar name	No.	Cd uptakes/(μg/kg)	Average value/(μg/kg)
First cluster/ low-Cd accumulated cluster	Huangjin	1	15.24±0.98	19.71
	Aikangjiaxue	3	19.86±2.05	
	Xiaobaicai 536	5	20.97±2.10	
	Bailingliren	9	22.77±0.86	
Second cluster/ medium-Cd accumulated cluster	Lvxiu	13	26.16±1.45	28.74
	Baixuegongzhu	20	31.32±2.41	
Third cluster/ high-Cd accumulated cluster	Tuzitui	21	37.65±0.88	40.74
	Jingpin	23	39.53±1.20	
	Degaoxialv	26	42.87±0.98	
	Nanhu 161	27	42.91±2.80	

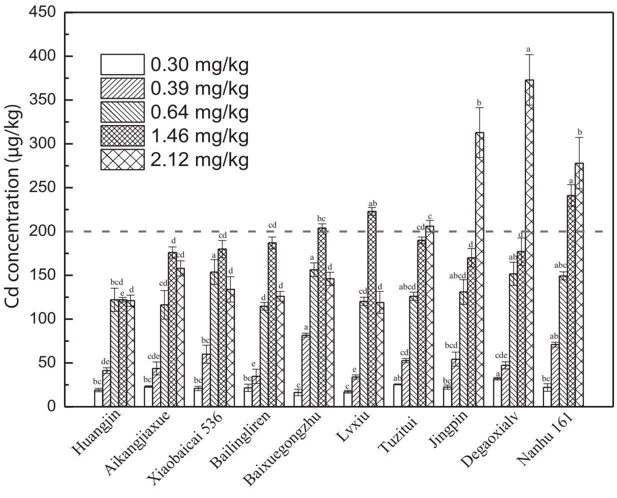

Fig. 3. Cd uptakes in edible parts of ten selected representative pakchoi cultivars under different Cd levels.

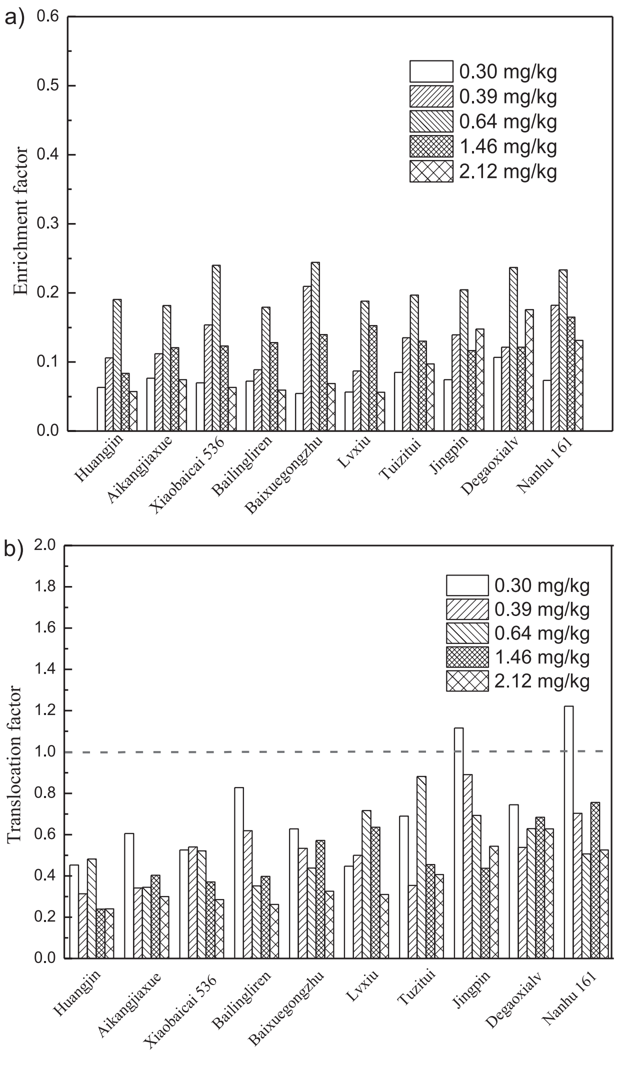

Fig. 4. a) EFs and b) TFs of ten selected representative pakchoi cultivars under different Cd levels.

of the Cd contaminated level in soil, the re-screening experiments in different Cd levels in contaminated soils were investigated, and the results are shown in Fig. 3. From Fig. 3, an obvious variability of Cd uptake can be seen among the ten selected pakchoi cultivars. All the ten selected pakchoi cultivars could be defined as “safe vegetables” when the Cd concentration in soil was below 0.64 mg/kg, but none of them belonged to “no-polluted vegetables.” However, when the Cd concentration in soil lowered to 0.39 mg/kg, even 40% of them could be seen as “no-polluted vegetables,” and among them, 75% belonged to the first cluster. Cd uptakes increased with the increasing Cd concentrations in soils, and the trend was most clear for the selected pakchoi cultivars in the third cluster. With the Cd concentration in soil exceeding 2.12 mg/kg, the values of Cd uptake in all selected pakchoi cultivars in the third cluster were beyond the standard of “safe vegetables,” while that in the first cluster still kept to a lower value. And there was no significant discrepancy of Cd uptake values under different Cd levels for the first cluster, further confirming the consistency and genotypic stability of the low Cd accumulating traits of the potential Cd-PSCs, and that these cultivars have high tolerance to Cd toxicity, which seemed to be a genetic connection [35].

To understand the abilities of Cd accumulation of ten representative pakchoi cultivaters and the translocation laws of Cd from roots to edible parts, the values of EFs and TFs were calculated (shown in Fig. 4), which were important in screening and breeding Cd-PSCs and then in minimizing the soil-to-plant transfer and roots-to-edible parts of Cd. It was clear from Fig. 4 that there were great differences both in EFs and TFs among the ten pakchoi cultivars. All selected representative pakchois had EFs less than 1.0 under the five Cd levels of contaminated soils. However, only 8 of the 10 selected representative pakchois had TFs less than 1.0 under the five Cd levels, including Huangjin, Aikangjiaxue, Xiaobaicai 536, Bailingliren, Baixuegongzhu, Lvxiu, Tuizitui, and Degaoxialv. And among them, 100% of pakchois in the first cluster had the potential to become Cd-PSCs under the five Cd levels according to this standard.

Conclusions

In this paper, a combination of primary screening experiments and re-screening experiments was conducted in field culture to systemic screening for Cd-PSCs from 30 selected pakchoi cultures. Taking all experimental results, including the results of cluster analysis performed on data from primary screening experiments and the re-screening experiments about the effects of the different Cd concentrations in contaminated soils on Cd uptakes and the values of EFs and TFs into consideration, Huangjin, Aikangjiaxue, Xiaobaicai 536, and Bailingliren in the first cluster as selected by cluster analysis could be confirmed as Cd-PSCs under the five investigated Cd levels with Cd uptake below 200 μg/kg, and the low Cd accumulating traits of the potential Cd-PSCs possess good genotypic stability.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. MAJOLAGBE A.O., ADEYI A.A., OSIBANJO O., ADAMS A.O., OJURI O.O. Pollution vulnerability and health risk assessment of groundwater around an engineering Landfill in Lagos, Nigeria. Chemistry International. **3**, 58, **2017**.
2. FAIT S., FAKHI S., ElMZIBRI M., FAIZ Z., FOUGRACH H., BADRI W., SMOUNI A., FAHR M. Distribution of metallic trace elements (ETM) in surface soils around the mediouna discharge (southern of Casablanca). Chemistry International. **3**, 278, **2017**.
3. MAJOLAGBE A.O., ADEYI A.A., OSIBANJO O. Vulnerability assessment of groundwater pollution in the vicinity of an active dumpsite (Olusosun), Lagos, Nigeria. Chemistry International. **2**, 232. **2016**.
4. IQBAL A., TABINDA A., YASAR A, MAHFOOZ Y. Heavy metal uptake and toxicity in tissues of commercially important freshwater fish (labeo rohita and wallago attu) from the Indus river, Pakistan. Polish Journal of Environmental Studies. **26** (2), 627, **2017**.
5. LI Z., MA Z., VANDER KUIJP T., YUAN Z., HUANG L. A review of soil heavy metal pollution from mines in China: pollution and health risk assessment. Science of the Total Environment. **468-469**, 843, **2014**.
6. MALANDRINO M., ABOLLINO O., BUOSO S., GIACOMINO A., GIOIA C., MENTASTI E. Accumulation of heavy metals from contaminated soil to plants and evaluation of soil remediation by vermiculite. Chemosphere. **82** (2), 169, **2011**.
7. SUN Y., LI Y., XU Y., LIANG X., WANG L. In situ, stabilization remediation of cadmium (Cd) and lead (Pb) co-contaminated paddy soil using bentonite. Applied Clay Science. **s105-106**, 200, **2015**.
8. LU K., YANG X., GIELEN G., BOLAN N., OK Y., NIAZI S., XU. S., YUAN G., CHEN X., ZHANG X., LIU D., SONG Z., LIU X., WANG H. Effect of bamboo and rice straw biochars on the mobility and redistribution of heavy metals (Cd, Cu, Pb and Zn) in contaminated soil. Journal of Environmental Management. **186** (Pt 2), 285, **2017**.
9. ADILOĢLU S. Using phytoremediation with canola to remove cobalt from agricultural soils. Polish Journal of Environmental Studies. **25** (6), 2251, **2016**.
10. WAN X., LEI M., CHEN T. Cost-benefit calculation of phytoremediation technology for heavy-metal-contaminated soil. Science of the Total Environment. **s563-564** (563), 796, **2016**.
11. BECH J., ROCA N., TUME P., RAMOS-MIRAS J., GIL C., BOLUDA R. Screening for new accumulator plants in heavy metal polluted soil surrounding Peruvian mine tailings. Catena. **136**, 66, **2015**.
12. SHARMA S., SINGH B., MANCHANDA V. Phytoremediation: role of terrestrial plants and aquatic macrophytes in the remediation of radionuclides and heavy metal contaminated soil and water. Environmental Science and Pollution Research. **22** (2), 946, **2015**.
13. BELLITURK K., SHRESTHA P., GÖRRES J. The importance of phytoremediation of heavy metal contaminated soil using vermicompost for sustainable agriculture. Journal of Endocrinology. **3** (3), 283, **2015**.
14. GILL S., KHAN N., ANJUM N., TUTEJA N. Amelioration of cadmium stress in crop plants by nutrients management: morphological, physiological and biochemical aspects. Plant Stress. **5** (s1), 1, **2011**.
15. SARKAR A., RAVINDRAN G., KRISHNAMURTHY V. A brief review on the effect of cadmium toxicity: from cellular to organ level. International Journal of Bio-Technology and Research. **3** (1), 17, **2013**.
16. YANG Q., XU Y., LIU S., HE J., LONG F. Concentration and potential health risk of heavy metals in market vegetables in Chongqing, China. Ecotoxicology & Environmental Safety. **74** (74), 1664, **2011**.
17. GILL S., KHAN N., TUTEJA N. Differential cadmium stress tolerance in five indian mustard (*Brassica juncea* L.) cultivars: an evaluation of the role of antioxidant machinery. Plant Signaling & Behavior. **6** (2), 293, **2011**.
18. LIU Y., ZHANG C., ZHAO Y., SUN S., LIU Z. Effects of growing seasons and genotypes on the accumulation of cadmium and mineral nutrients in rice grown in cadmium contaminated soil. Science of The Total Environment. **579** (1), 1282, **2016**.
19. 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 & Pollution Research International. **22** (11), 8432, **2015**.
20. YU H., WANG J., FANG W., YUAN J., YANG Z. Cadmium accumulation in different rice cultivars and screening for pollution-safe cultivars of rice. Science of the Total Environment. **370** (2), 302, **2006**.
21. ZHANG H., ZHANG X., LI T., HUANG F. Variation of cadmium uptake, translocation among rice lines and detecting for potential cadmium-safe cultivars. Environmental Earth Sciences. **71** (1), 277, **2014**.
22. LI B., HE W., WANG C., GUO Y., ZHANG J. Selecting for cadmium exclusion or low accumulation rice cultivars in slight-moderate pollution area under field conditions. Polish Journal of Environmental Studies. **23** (4), 1347, **2014**.
23. WANG J., YU N., SHINWARI K., SHEN Z., ZHENG L. Screening for Cd-safe cultivars of Chinese cabbage and a preliminary study on the mechanisms of Cd accumulation. International Journal of Environmental Research & Public Health. **14** (4), 395, **2017**.

24. CLARKE J., MCCAIG T., DEPAUW R., KNOX R., CLARKE. F., FERNANDEZ. M., AMES. N. Registration of 'Strongfield' durum wheat. Crop Science. **46** (5), 253, **2006**.
25. CAO F., WANG R., CHENG W., ZENG F., AHMED I., 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**.
26. SUNGUR A., SOYLAK M., ÖZCAN H. Chemical fractionation, mobility and environmental impacts of heavy metals in greenhouse soils from Çanakkale, Turkey. Environmental Earth Sciences. **75** (4), 334, **2016**.
27. RIVERA M.B., GIRÁLDEZ M.I., FERNÁNDEZ-CALIANI J.C. Assessing the environmental availability of heavy metals in geogenically contaminated soils of the Sierra de Aracena Natural Park (SW Spain). Is there a health risk?. Science of the Total Environment. 560-561, 254, **2016**.
28. DONG W., CUI Y., LIU X. Instances of soil and crop heavy metal contamination in China. Soil & Sediment Contamination. **10** (5), 497, **2001**.
29. WANG L., XU Y., SUN Y., LIANG X., LIN D. Identification of pakchoi cultivars with low cadmium accumulation and soil factors that affect their cadmium uptake and translocation. Frontiers of Environmental Science & Engineering. **8** (6), 877, **2014**.
30. MMOLAWA K., LIKUKU A., GABOUTLOELOE G. Assessment of heavy metal pollution in soils along major roadside areas in Botswana. African Journal of Environmental Science & Technology. **5** (3), 186, **2011**.
31. YADAV S., CHANDRA R. Heavy metals accumulation and ecophysiological effect on *Typha angustifolia* L. and *Cyperus esculentus* L. growing in distillery and tannery effluent polluted natural wetland site, Unnao, India. Environmental Earth Sciences. **62** (6), 1235, **2011**.
32. CHEN Y., LI T., HAN X., DING Z., YANG X., JIN Y. Cadmium accumulation in different pakchoi cultivars and screening for pollution-safe cultivars. Journal of Zhejiang University Science B. **13** (6), 494, **2012**.
33. HU J., WU F., WU S., SUN X., LIN X., WONG M. Phytoavailability and phytovariety codetermine the bioaccumulation risk of heavy metal from soils, focusing on Cd-contaminated vegetable farms around the Pearl River Delta, China. Ecotoxicology & Environmental Safety. **91** (2), 18, **2013**.
34. BENABDALLAH N.K., HARRACHE D., MIR A., GUARDIA M.D.L., BENHACHEM F.Z. Bioaccumulation of trace metals by red alga Corallina elongata in the coast of Beni Saf, west coast, Algeria. Chemistry International. **3**, 220, **2017**.
35. MA M., LAU A., JIA Y., TSANG W., LAM S., TAM N., WONG Y. The isolation and characterization of Type 1 metallothionein (MT) cDNA from a heavy-metal-tolerant plant, Festuca rubra, cv. Merlin. Plant Science. **164** (1), 51, **2003**.