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PII: S0269-7491(19)37027-7

DOI: https://doi.org/10.1016/j.envpol.2020.114213

Reference: ENPO 114213

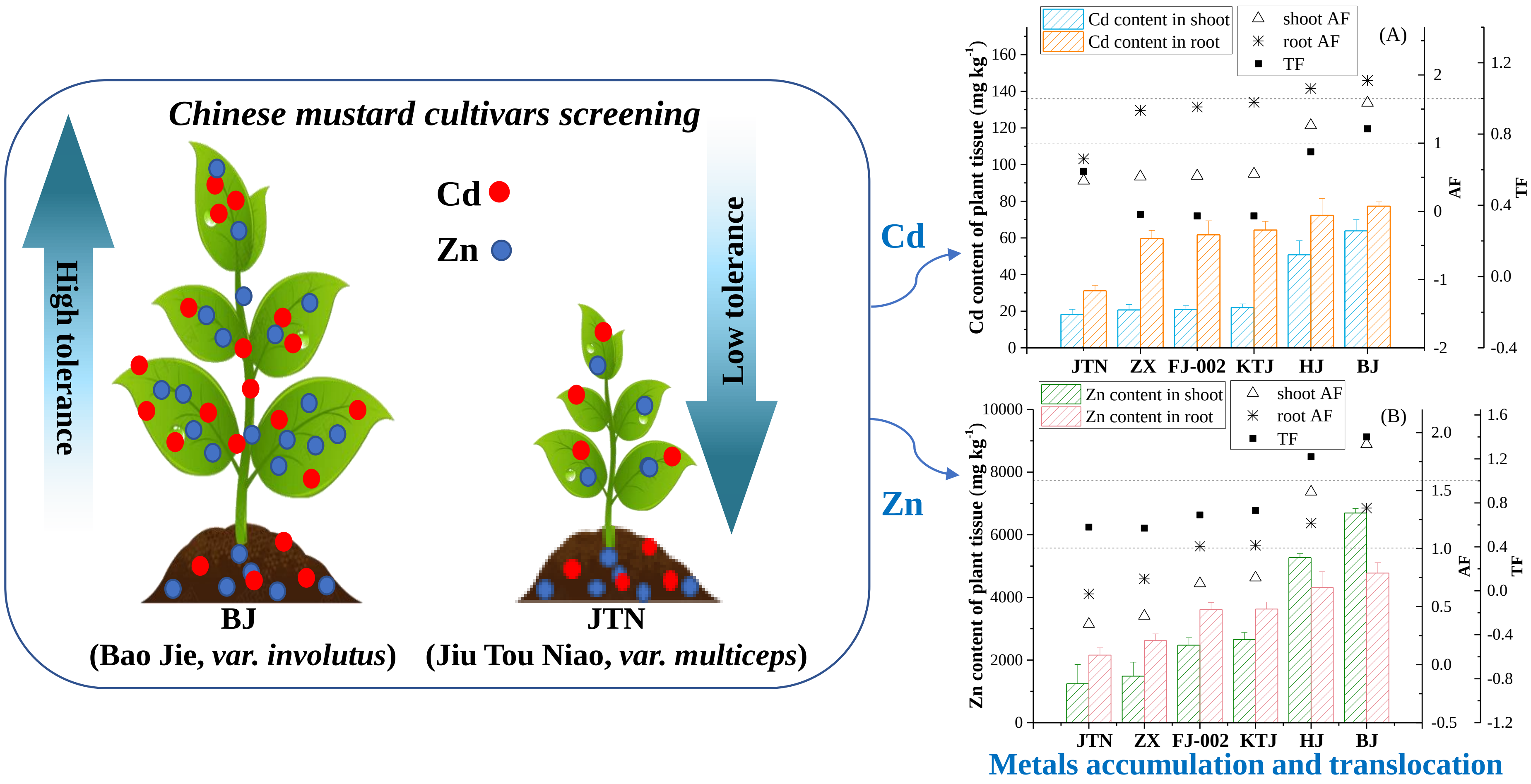
To appear in: *Environmental Pollution*

Received Date: 25 November 2019

Revised Date: 26 January 2020

Accepted Date: 16 February 2020

Please cite this article as: Du, J., Guo, Z., Li, R., Guo, D., Lahori, A.H., Wang, P., Liu, X., Wang, X., Ali, A., Zhang, Z., Screening of Chinese mustard (*Brassica juncea* L.) cultivars for Cd/Zn phytoremediation and research on physiological mechanisms, *Environmental Pollution* (2020), doi: https://doi.org/10.1016/j.envpol.2020.114213.


Chinese mustard cultivars screening
High tolerance
Low tolerance
Cd
Zn
BJ
(Bao Jie, var. involutus)
JTN
(Jiu Tou Niao, var. multiceps)
Cd
Zn
Cd content in shoot
Cd content in root
shoot AF
root AF
TF
(A)
Cd content of plant tissue (mg kg-1)
AF
TF
0
20
40
60
80
100
120
140
160
-2
-1
0
1
2
-0.4
0.0
0.4
0.8
1.2
JTN
ZX
FJ-002
KTJ
HJ
BJ
Zn content in shoot
Zn content in root
shoot AF
root AF
TF
(B)
Zn content of plant tissue (mg kg-1)
AF
TF
0
2000
4000
6000
8000
10000
-0.5
0.0
0.5
1.0
1.5
2.0
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
JTN
ZX
FJ-002
KTJ
HJ
BJ
Metals accumulation and translocation

Title page：

Screening of Chinese mustard (*Brassica juncea* L.) cultivars for Cd/Zn phytoremediation and research on physiological mechanisms

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Abstract

To identify the potential Cd/Zn accumulators with high tolerance among Chinese mustard (*Brassica juncea* L.) cultivars, a pot experiment involving elevated Cd/Zn exposure concentrations (0.12–40.27 mg kg^{-1} for Cd and 136.4–3530 mg kg^{-1} for Zn) was performed among 21 cultivars. Regarding physiological and biochemical indicators (such as biomass, chlorophyll and antioxidants) under Cd/Zn stress, principal component analysis (PCA) and cluster analysis (CA) were used for cultivar tolerance evaluation and classification. Results showed that BJ (Baojie, *var. involutus*) cultivar was distinguished as a potential phytoremediation candidate from other cultivars, which had the highest Cd/Zn tolerance, remarkable accumulation and translocation capacity (biological concentration factor (BCF) >1 for Cd and Zn; translocation factor (TF) >0.8 for Cd and TF>1 for Zn). Additionally, the antioxidant enzymes played a protective role against ROS (reactive oxygen species) under low Cd/Zn stress, whereas the defense system might be collapsed under relatively high Cd/Zn stress. The investigation results indicated that BJ (Baojie, *var. involutus*), as a native cultivar, can be further applied in soil remediation.

Keywords: Phytoremediation; Screen; Tolerance; Antioxidant enzymes

1. Introduction

Recently, soil contamination caused by potentially toxic metals (PTMs) has been a worldwide environmental issue, which is mainly due to the anthropogenic activities, such as mining and smelting (Sidhu et al., 2017). The constant accumulation of PTMs in soil could pose severe risk to living organisms including plants, animals and microorganisms (Zhang et al., 2015). To date, phytoremediation is confirmed to be the most environmentally friendly and cost-effective strategy, in which phytoextraction refers to the use of accumulator species to remove PTMs from soil via root uptake and root-shoot translocation (Wu et al., 2018; Yang et al., 2018).

Nowadays, tens of thousands of PTMs accumulators have been identified around the world, among which Indian mustard (*Brassica juncea* L., *Czern.* and *Coss.*) was an attractive hyperaccumulator ascribing to its high tolerance under PTMs stress, great accumulation capacity of metals and large biomass (Mobin and Khan, 2007). However, Indian mustard is sensitive to climate and soil condition change, thus such species might not be widely applied in remediation practice in China. Accordingly, Chinese mustard (*Brassica juncea* L.), affiliated to the identical family with Indian mustard, was supposed to have the similar superiority in phytoremediation. It is known to all that there are a large variety of Chinese mustard cultivars distributed in different areas of China, most of which have great geographical and climatic adaptability. Moreover, Chinese mustard cultivars have other features such as large biomass, rapid growth rate, and can be cultivated several times every year during a relatively long and suitable planting period. In addition, the application of native plants in contaminated soil would make little disturbance to the soil and meanwhile reduce the expense on replanting, mowing and harvesting (Marrugo-Negrete et al., 2016). Therefore, Chinese mustard was probably more economical and practical in phytoremediation application. However, few investigations have concentrated on the screening of Chinese mustard for phytoremediation application. Thus, in order to acquire a comprehensive knowledge of the possibility of different Chinese mustard cultivars in soil restoration and to screen promising phytoremediation candidates, 21 kinds of Chinese mustard cultivars obtained from different districts of China were studied in present work for their phytoextraction potential. Consequently, the main objectives of this research were to (1) study the growth response of 21 Chinese mustard cultivars under different Cd/Zn gradient stress, (2) screen the Chinese mustard cultivar with the highest Cd/Zn tolerance referring to a variety of indicators via principal component analysis, (3) evaluate the accumulation and translocation capacity of the representative cultivars, and (4) demonstrate the tolerance mechanisms via analyzing the physiological and biochemical indicators of the representative cultivars.

2. Materials and methods

2.1. Soil characterization and pot experiment design

The soils prepared for pot experiment were sampled from six areas in Feng County (33°34′57″-34°18′21″ E, 106°24′54″-107°7′30″ N) of Shaanxi Province, China. The selected soil sampling sites were suffering different degrees of PTMs (Cd and Zn) pollution from mining and smelting activities in this area. The detailed description about the PTMs contaminated areas of Feng County have been presented in our previous work (Ali et al., 2017; Shen et al., 2017; Xiao et al., 2018). Six Cd/Zn treatments (T0-T5) were designed for pot experiment using the six sampling soils, respectively. The soil physicochemical properties of T0-T5 treatments were shown in Table S1. The total Cd contents of soils in T0-T5 treatments were 0.12, 1.14, 3.72, 6.65, 11.83 and 40.27 mg kg^{-1}, respectively. The total Zn contents of soils in T0-T5 treatments ranged from 136.4–3530 mg kg^{-1}. T0 treatment was served as a control with Cd/Zn concentrations lower than the limited values of Soil environmental quality-Risk control standard for soil contamination of agricultural land (State Standard of the People’s Republic of China GB15618-2018). The description of 21 Chinese mustard (*Brassica juncea* L.) cultivars prepared for the pot experiment was specified in Table S2.

The pot experiment was conducted in a greenhouse at Northwest A&F University, Yangling, China (34°15′49″ N, 108°3′42″ E). Twenty seeds were uniformly sown in each pot and thinned to 5 seedlings after the third leaf emerging. All pots were supplemented with 200 ml tap water every two days to maintain 70% of the field capacity. Chemical fertilizers were added to each pot to achieve the nutrient levels of 100 mg·kg^{-1} alkaline nitrogen, 120 mg·kg^{-1}available phosphorus and 350 mg·kg^{-1} available potassium in soils.

2.2 Samples analysis

2.2.1 Soil parameters measurement

The pH value was measured in the soil/water suspension (1:2, w/v) using a pH meter (Seven Compact, Mettler Toledo, Greifensee, Switzerland) (USEPA Method 9045D). The soil organic matter was determined as described by Mahar et al. (2016). The contents of alkaline nitrogen, available phosphorus and potassium were measured according to the Methods of Soil Analysis (Page et al., 1982). As for total PTMs concentration analysis, 0.5 g soil samples were digested with HNO_3-H_2O_2-HF (3:1:1, v/v/v) mixture in a microwave digestion apparatus (ETHOS, America) (USEPA Method 3051A). The Cd/Zn concentrations in the digested samples were then determined by a flame atomic absorption spectrometer (FAAS) (Z-2000, Hitachi, Japan). Diethylene triamine pentaacetate acid (DTPA) extraction method (NY/T 890-2004, Ministry of Agriculture of the People's Republic of China) was used to estimate the metal bioavailability.

2.2.2 Plant physiological and biochemical measurement

All plants have grown for a period of 60 days. Prior to harvest, five mature leaves from each pot were picked and cryopreserved for fresh sample determination. Subsequently, the exchangeable Cd/Zn ions attaching to the roots surface were removed by immersing the roots in EDTA-2Na solution (15 mM) for 30 min. All parts of the plants were washed by deionized water finally. The lengths of shoot and root, and the fresh weights (FW) of shoot and root were all recorded. Then, the clean samples were dried in oven till a constant weight (80 °C), and then the dry weights (DW) of shoot and root biomass were measured. Afterwards, the dry shoots and roots were milled into powder (< 0.15 mm), respectively, and preserved in sealed bags for further processing.

The relative chlorophyll content of leaves was measured by a portable chlorophyll meter (SPAD-502, Minolta, Japan; SPAD, Soil and Plant Analyzer Development). Lipid peroxidation was assessed by determining the malonaldehyde (MDA) content of plant tissue according to Heath and Packer (1968). In brief, fresh leaves (0.5 g) were ground and extracted by 10 ml of phosphate buffer (1 mM ethylenediaminetetraacetic acid disodium salt and 50 mM potassium phosphate, pH 7.8) under 4 °C environment (Guo et al., 2019). The enzymatic activities of the plant tissue, such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), were determined by a spectrophotometer (UV-752N, JINGKE, China). All enzymatic activity data were presented as U g^{-1} fresh weight (FW) (Aebi, 1984; Zhang et al., 2013).

2.3. Index calculation

Tolerance index (TI) referrs to the ratio of growth index (the average value of shoot length, root length, shoot biomass and root biomass) of metal-loaded plant tissue to that of the clean plant tissue (control). The detailed calculation method has been reported by Wu et al. (2018). Biological concentration factor (BCF) and translocation factor (TF) were used to assess the PTMs accumulation capacity and the transportation ability of plant species for soil remediation. BCF is defined as the ratio of metal concentration in plant shoots/roots to that in the soil. TF is the ratio of metal concentration translocated in shoots to that presented in roots of the plants (Sidhu et al., 2017).

2.4. Quality control and statistical analysis

All analyses were conducted in triplicates, and reagent blanks were used to correct the determination results. Standard soil and plant reference substances (National Research Center for Standards of China, GBW07405 and GBW07602) were supplied for quality assurance. Statistical analysis was performed using SPSS 23.0 software. The physiological and biochemical parameters of the cultivars were subjected to cluster analysis (CA) and principal component analysis (PCA). Principal component analysis was carried out to identify the latent factors (principal components, PCs), and then the extracted components were used for tolerance comprehensive evaluation and cultivars screening. Cluster analysis was performed to classify the cultivars following the Ward’s algorithmic method and the distances between the cultivars were calculated according to the method of square Euclidean distances (Jin et al., 2019; Liang et al., 2013). All the figures were drawn by Origin pro software (version 2016).

3. Results and discussion

3.1. Growth response to Cd/Zn exposure

The growth indicators and tolerance index (TI) of the 21 Chinese mustard cultivars exposing to different degrees of Cd/Zn stress were displayed in Table S3 and Fig.1. The existence of Cd/Zn significantly affected the mustard growth in T1-T5 compared with the control in T0. It was noteworthy that low Cd/Zn stress (T1) acted on promoting the growth of most cultivars (except JTN (Jiutouniao, *var. multiceps*) and XXJ (Xixuejie, *var. multiceps*)); especially for BJ (Baojie, *var. involutus*). The shoot height, root length, shoot and root biomass of BJ were increased by 29.0, 12.2, 17.8 and 28.5% in T1, respectively, with respect to that in T0. Accordingly, the TI values of most cultivars in T1 exceed 1, indicating that a low metal concentration could simulate the plant growth which was consistent with the results reported previously (Jia et al., 2013; Jia et al., 2015; Sidhu et al., 2017; Wu et al., 2018). Nevertheless, with the increasing Cd/Zn content (T2-T5), the inhibitory effects of Cd/Zn-induced stress on mustard growth were more and more serious. Among the 21 cultivars, the inhibition of the growth of JTN was the most obvious that its shoot height, root length, shoot and root biomass under T2-T5 were decreased by 28.1–67.7%, 42.0–73.3%, 3.7–76.8% and 11.8–83.3%, respectively, compared with that in T0. The TI values of almost all cultivars under T2-T5 were less than 1 and decreased with the enhancing metal stress. Notably, the TI were no more than 0.6 in T5, in which BJ presented the maximum TI of 0.56 and JTN the lowest one of 0.25. Thus, referring to the plant growth, BJ has the highest tolerance and JTN was the most vulnerable cultivar whose TI was the lowest independent of the treatments.

The plant growth promotion under low metal stress and the growth inhibition under high metal stress suggested the occurrence of hormesis effect. The positive effect of low dose metal on growth enhancement might be associated with the increase in photosynthetic carbon assimilation, and a high net photosynthesis rate would help in facilitating the gas exchange and transpiration of plant leaves and increasing the photosynthetic pigments (Jia et al., 2015). Under low Cd exposure, Sidhu et al. (2017) explained that the toxic metal might be detained in the non-metabolic parts of plant such as the cell wall and vacuole, thus alleviating or preventing the toxic effects on plant growth and metabolism. However, the growth inhibition response to high doses metal exposure can ascribe to the deficiency in nutrient uptake, the limitation of cell development resulting from the root metabolic activity depress and the cell wall lignification (Finger-Teixeira et al., 2010). Additionally, the metal-induced stress might result in the reduction of photosynthetic carbon assimilation of the plant aerial parts and thereby inhibit the plant growth (Redondo-Gómez et al., 2010). The knowledge of the plant hormesis effect under metal stress could provide a reference for biomass production and effective phytoremediation practice.

[Fig. 1]

3.2. Multivariate analysis

The multivariate analysis was preformed referring to 11 variables of mustard such as plant root dry weight (RDW), shoot dry weight (SDW), shoot height (SH), root length (RL), chlorophyll content (SPAD), shoot fresh weight (SFW), root fresh weight (RFW), malonaldehyde (MDA), superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in T5 (Table S4).

3.2.1. Principal component analysis

Table S5 showed the correlation analysis results of the above 11 variables. Strong positive correlation (coefficients > 0.75) was observed among the plant growth indicators, such as RDW, SDW, SFW, SH and RL. There were also strong positive correlations (coefficients > 0.75) among SPAD, SOD, POD and the plant growth indicators expect RFW (coefficients ~0.5). However, significant negative correlation (coefficients < -0.8) was found among MDA and RDW, SDW, SFW, SH, RL, RDW, SDW, SFW, SH and RL. Additionally, no significant correlation between CAT and other indicators was observed. Further discussion on the relationship among the variables was provided in Section 3.4. On the other hand, the strong correlation among the above variables indicated that the information provided by them might overlap with each other, and the role of each individual index was different in mustard tolerance evaluation. Thus, principal component analysis (PCA) was conducted to simplify data array by converting original multivariate variables into several new and unrelated ones, which were given the name principle components with less information loss (Wesołowski & Konieczynski, 2003).

The principle components (PC) extraction results were shown in Table S6. Three significant components were derived, accounting for 91.8% of the total variance. The first component was highly loaded by RDW, SDW, SFW, SH, RL, SPAD, SOD and POD; whereas the negative loading of MDA in PC1 indicated the antagonistic effect with respect to plant growth. The greatest contribution to PC2 was associated with CAT (0.871), followed by SOD (0.227), and other variables (less than 0.1). The third component was dominated by RFW (0.498) and followed by CAT (0.402) and RDW (0.267). This presentation was greatly supported by the three-dimensional (PC1 versus PC2 versus

PC3) plotting of the loadings in Fig.2 (A). According to the PCA extraction results, the comprehensive index and membership function values (Table 1) for tolerance assessment were calculated in terms of the feature vector of PC1, PC2 and PC3 (Table S7) and the measured value of variables (Table S4). Furthermore, the sum of the three membership function values multiplied by contribution rate of PC1, PC2 and PC3 was defined as value D, which was used for mustard cultivars sorting. With respect to the value D shown in Table 1, the tolerance of 21 Chinese mustard cultivars followed the order: BJ > HJ > TJ-381 > WJ > JT-F1 > BJ-3111 > DJ > KTJ > SY-16 > FJ-002 > BJ-338 > SJ > CC-602 > FJ-388 > JXJ > TJ-391 > CC > XXJ > JSC > ZX > JTN (The abbreviation names of the 21 cultivars were specified in Table S2). Obviously, BJ (Baojie, *var. involutus*) was evaluated to have the greatest tolerance with the highest score of 0.904, whereas JTN (Jiutouniao, *var. multiceps*) might have the lowest tolerance with the value of 0.087.

[Table 1]

3.2.2. Cluster analysis

A dendrogram obtained from cluster analysis, referring to the comprehensive evaluation index (value D) of the 21 cultivars, was shown in Fig. 2 (B). The abscissa axis represented the degree of tolerance difference among the cultivars; i.e., the higher value on distance axis, the more significant the difference is. As to the distance value of 5, the dendrogram can be divided into four main clusters: cluster 1 (BJ), cluster 2 (HJ, WJ and TJ-381), cluster 3 (JXJ, BJ-3111, FJ-002, SY-16, SJ, FJ-388, DJ, KTJ, JT-F1, BJ-388 and CC-602) and cluster 4 (JTN, JSC, CC, TJ-391, ZX and XXJ). Furthermore, cluster 3 can be divided into two sub clusters (subcluster 1: KTJ, DJ, SY-16, JT-F1 and BJ-3111; subcluster 2: BJ-388, SJ, FJ-002, CC-602, FJ-388 and JXJ). According to the distance between the groups, the difference between cluster 1 and 2 was not much significant, so was cluster 3 and 4; whereas, the difference between cluster 1 and 4 was considerably significant. The relatively large distance between cluster 1 and 4 indicated that the tolerance of the two groups was obviously different. It was notable that the classification result in Fig. 2 (B) was consistent with the sorting result in Table 1. For example, BJ belonging to cluster 1 had the highest value D, and was sorted as 1; while TJ-391, CC, XXJ, JSC, ZX and JTN belonging to cluster 2, sorting as 16, 17, 18, 19, 20 and 21, respectively, in terms of their value D. Thus, according to the tolerance sorting results and the cluster analysis, cluster 1, 2, 3 and 4 could represent the groups that have the highest, higher, medium and lowest tolerance under Cd/Zn stress, respectively.

[Fig. 2]

3.2.3 Regression Analysis

Regression analysis was conducted in this work to screen effective tolerance indexes, and to establish accurate mathematical model for Cd/Zn tolerance prediction. A stepwise regression analysis was performed for the 11 tolerance indices of the 21 mustard cultivars. In the regression analysis, the comprehensive evaluation value of Cd/Zn tolerance (value D) was the dependent variable, and all the 11 tolerance indexes were used as independent variables. After regression analysis, the optimal regression equation for the Cd/Zn tolerance of Chinese mustard can be expressed as follows:

$$D = 0.7212 + 0.5283X_1 + 0.2592X_2 - 0.1751X_8 + 0.3462X_{11}$$

where D is the comprehensive evaluation value of Cd/Zn tolerance, X_1 is the root dry weight (RDW),

X_2 is the shoot dry weight (SDW), X_8 is the malonaldehyde (MDA) and X_{11} is the catalase (CAT).

The predicted deviation of the above regression equation was from -10.83% to 5.57% and the correlation coefficient R between the equation predicted D value and the actual D value was as high as 0.9995, both of which proved the reliability and accuracy of the regression equation. According to the equation, among the 11 indexes, the above four (X_1, X_2, X_8, and X_{11}) were closely related to the Cd/Zn tolerance of Chinese mustard. Using this equation could significantly simplify the work of evaluating or predicting mustard Cd/Zn tolerance.

3.3. Cd/Zn accumulation and translocation

BJ and JTN were identified as the cultivars with the strongest and the weakest tolerance under Cd/Zn stress. As the representative cultivars, the correlation between Cd/Zn content in soils (T0, T1, T2, T3, T4 and T5) and that in shoots and roots (BJ and JTN) were shown in Fig.3. Notably, the Cd/Zn content of plant tissue (shoot and root) increased with the ascending Cd/Zn exposure concentration from T0 to T5, and a positive linear correlation was observed between the Cd/Zn concentration in plant tissues and that in the soil ($r^2 > 0.9$, $p < 0.05$), which exerted a dose-response relationship (Zhou et al., 2017). Thus, the accumulated amount of Cd/Zn in the mustard tissue was directly associated with the content of Cd/Zn in the soil (Marrugo-Negrete et al., 2016; Sidhu et al., 2017). BJ and JTN accumulated the maximum Cd of 63.85 and 20.71 mg kg^{-1} DW in shoots, 77.29 and 31.17 mg kg^{-1} DW in roots, respectively, when exposed to the highest Cd concentration in T5. The maximum Zn uptake by BJ and JTN were 6693 and 1245 mg kg^{-1} DW in shoots, 4777 and 2622 mg kg^{-1} DW in roots, respectively, under the highest Zn stress in T5. The accumulation of Cd/Zn in BJ plant tissue was much higher than that in JTN.

Fig. 4 displayed the Cd/Zn content, BCF and TF values of shoots/roots with respect to the cultivars of JTN, ZX, FJ-002, KTJ, HJ and BJ in T5, which have presented the distinctive tolerance under metal stress according to the screening results in section 3.2 (JTN < ZX < FJ-002 < KTJ < HJ < BJ). The Cd and Zn concentration of JTN, ZX, FJ-002, KTJ, HJ and BJ ranged from 18.26 to 63.85 mg kg^{-1} DW and 1245 to 6693 mg kg^{-1} DW in the shoots, 31.17 to 77.29 mg kg^{-1} DW and 2158 to 77.29 mg kg^{-1} DW in the roots, respectively. Obviously, BJ had extracted the maximum Cd/Zn amount, and JTN extracted the minimum. This accumulation behavior indicated that the Cd/Zn extraction in shoots/roots of the six representative cultivars increased with their elevated tolerance under Cd/Zn stress. The BCF values of Cd/Zn presented the following order: JTN < ZX < FJ-002 < KTJ < HJ < BJ, among which the Cd/Zn shoot BCF values of HJ and BJ exceed 1. Additionally, the root BCF values of Cd for all the cultivars were greater than 1 except JTN (0.77), and that of Zn were mostly greater than 1 except JTN (0.61) and ZX (0.74). The BCF >1 demonstrated the potential ability of plant cultivars for phytoremediation (Liu et al., 2010; Wu et al., 2018). All TF values of Cd for the six cultivars were below 1, suggesting the restricted translocation of Cd from underground to aerial parts. Still, the highest TF value (0.83) was observed for BJ, and followed by HJ (0.70). With respect to the TF values of Zn, only BJ and HJ were above 1, and were recorded 1.40 for BJ and 1.22 for HJ, respectively. It was notable that the studied cultivars transported more Zn from shoots to roots as compared to Cd, indicating a more efficient translocation of Zn in plant tissues.

With respect to the Cd/Zn accumulation of the mustard cultivars, metals preferred to concentrate in the roots rather than the shoots in most cases. Root was the initial site for metal uptake/accumulation, and the free metal ions in the soil might be taken up by the underground part via water translocation and retained in the root, with limited transportation to the aerial part (Xiao et al., 2018; Zhou et al., 2017). Some specific strategies have been pronounced associated with the metal uptake from the soil. Zhang et al. (2014) suggested that Cd might enter the plant roots aided by the transporters of Fe and Mn due to the reduction of Fe accumulation in vetiver grass roots under enhanced Cd stress. Hart et al. (2002) documented the competitive interaction between Cd and Zn at the cell plasma membrane in the roots of wheat, deducing that the entry of them was generally through a common transporter/carrier and the affinity of carrier for Cd was greater than that of Zn. Furthermore, according to the Cd/Zn influx kinetic constant, when the affinity of carrier is higher for Cd, it would stimulate a relatively high Zn activity to hinder the Cd extraction. In addition, Liu et al. (2010) had presumed that Cd would directly enter the plant roots or via free diffusion in some chloro-complexed forms which were more bioavailable. After accumulation in the roots, metals could be translocated to the shoots by evapotranspiration or the metals transporting ATPases (Tangahu et al., 2011; Rascio and Navari-Izzo, 2011). However, a large proportion of metals was still retained in the roots and difficult to transport to the aboveground parts which could be ascribing to the strong binding of metal ions to the cell wall or the retention in the vacuoles of the roots (Yang et al., 2018; Zhang et al., 2015).

[Fig. 3]

[Fig. 4]

3.4. Physiological and biochemical indicators

With the accumulation of Cd/Zn in plant tissues, the mustard cultivars would undergo a variety of physiological and biochemical changes. Several indicators, e.g., SPAD, MDA, SOD, CAT, POD values of the 6 representative mustard cultivars (JTN, ZX, FJ-002, KTJ, HJ and BJ) under different

Cd/Zn stress were shown in Fig S1. SPAD value was widely used to estimate the relative content of chlorophyll, which was regarded as an indicator of plant photosynthesis rate. In general, the SPAD value of the mustard cultivars decreased with the elevated Cd/Zn exposure concentration, except ZX, KTJ, HJ and BJ in T1, comparing to the control. Similar results have been reported by Yang et al. (2018) that the chlorophyll content of *K. paniculata* seedlings increased firstly, and then decreased with the Cd stress enhancement. The increased SPAD value under low Cd/Zn exposure concentration may involve the similar mechanism associated with hormesis effect as discussed in section 3.1. Under high Cd/Zn stress, more toxic metals would combine with the mercapto group of chloroplasts, simultaneously damage the enzyme activity for chlorophyll photosynthesis, thereby hindering the chlorophyll synthesis (Jiang et al., 2007). Additionally, toxic metals could also lead to the increase in plastogobuli and thylakoid membranes damage in the chloroplast, accelerating leaf cell aging (Jin et al., 2008).

MDA, as the final product of membrane lipid peroxidation, would accumulate in plant and result in severe damage to the cells when plants are suffering oxidative stresses. Thus, the MDA could act as an indicator of lipid peroxidation and is applied to assess the oxidative damage of plants resulted from metal stress (Guo et al., 2014; Li et al., 2013). In present study, the gradually enhanced Cd/Zn exposure led to an increase of MDA content in the leaves of all six mustard cultivars, indicating that Cd/Zn had induced more severe cell membrane oxidative stress and higher degree of lipid peroxidation. This would further cause adverse effect on the cell membrane, chloroplast, mitochondria and other organelles, and thus inhibit the normal physiological growth of plants (Celekli et al., 2013). Moreover, it was noticeable that BJ had the minimum MDA content independent of the treatments, demonstrating that it had the highest tolerance against Cd/Zn stress as compared with other cultivars.

Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), could play a major part in protecting the plant from reactive oxygen species (ROS) injury which resulted from lipid peroxidation under toxic metals stress (Li et al., 2016; Sidhu et al., 2017). As shown in Fig. 5, SOD and CAT activities of FJ-002, KTJ, HJ and BJ, and POD activities of all 6 mustard cultivars increased initially under low Cd/Zn stress as compared to controls and then declined dramatically with the enhanced metals exposure concentration. Previous studies had displayed similar variation trend for *Hibiscus cannabinus* L., *Koelreuteria paniculate* and *Phyllostachys pubescens*, respectively, under Cd-induced oxidative stress (Li et al., 2013; Li et al., 2016; Yang et al., 2018). This can be explained that the defense system established by antioxidant enzymes could probably scavenge the ROS caused by toxic metals stress (in T2 and T3) and thereby ensure the normal metabolism of plants. However, when the Cd/Zn induced stress enhanced to a relatively high degree (in T4 and T5), the activities of SOD, CAT and POD were observed a rapidly decrease, demonstrating that the generated ROS in plants might overwhelm the defense ability of the antioxidant enzymes or perhaps the toxic metals bound to the enzymes active centers (Sidhu et al., 2017). Thus, the adverse effects of toxic metals on plants tissues would occur again and might be accelerated with the increasing Cd/Zn stress, resulting in the inhabitation of antioxidant enzymes synthesis (Li et al., 2016; Yang et al., 2018). Additionally, among the 6 cultivars, BJ presented the highest SOD, CAT and POD activities in most treatments as compared with other cultivars, indicating BJ had the greatest scavenging ability of antioxidant enzymes and the strongest defense system against toxic metals. Besides, the SOD and CAT activities of JTN and ZX decreased gradually with the ascending Cd/Zn stress, which were in accord with their relatively low Cd/Zn tolerance as mentioned above.

The detoxification mechanisms of phytoremediation plants were mainly associated with the activities of antioxidant enzymes. SOD, an important component of antioxidant system, acts as the first defense line against lipid peroxidation to avoid ROS-oxidative damage. SOD can catalyze the dismutation of O_2^- to H_2O_2 and oxygen in the plant cells, and thereby eliminate the superoxide radicals, alleviating the lipid peroxidation of membrane and maintaining the cell membrane in a stable state. CAT is also an essential enzyme involved in scavenging of toxic peroxides; it can directly transform H_2O_2 into water and molecular oxygen, playing an important role in H_2O_2 elimination. POD also catalyzes H_2O_2-dependent oxidation of the substrate. Additionally, POD is involved in lignin biosynthesis and constructing a mechanical barrier in plant tissues against toxic metals stress (Krantev et al., 2008). Therefore, the synthesis of antioxidant enzymes in plant tissue might be the involved mechanism for the establishment of defense system against ROS under toxic metals stress.

4. Conclusions

In present study, a systemic screening method was used to identify the Chinese mustard cultivars with the highest tolerance exposed to Cd/Zn stress. The pot experiment results showed the plant growth promotion under low Cd/Zn stress and the growth inhibition under high Cd/Zn stress, suggesting the occurrence of hormesis effect. The principal component analysis and the cluster analysis presented that the 21 Chinese mustard cultivars can be classified into 4 groups: the highest, higher, medium and lowest tolerance under Cd/Zn stress, among which BJ (Baojie, *var. involutus*) has the greatest tolerance whereas TJN (Jiutouniao, *var. multiceps*) the weakest. The Chinese mustard cultivars presented relatively high BCF and TF, especially for BJ and the Cd/Zn accumulation and translocation capacity of the representative cultivars increased with their elevated metals tolerance. MDA production in plant leaf tissues indicated the increasing oxidative stress with the enhanced Cd/Zn exposure concentration, and the lowest MDA value in BJ suggested the least impact of lipid peroxidation on it. The antioxidant enzymes (SOD, CAT and POD) played a protective role against ROS under low Cd/Zn stress, whereas the defense system would be collapsed under the gradually increasing Cd/Zn stress. According to the above screening results, BJ was identified as the most promising candidate for phytoremediation, and further research is required to promote the phytoremediation efficiency of Chinese mustard, such as chemical and biological assisted/combined remediation.

Acknowledgement

This work was financially supported by the Science and Technology Overall Innovation Project of Shaanxi Province, China (NO. 2016KTCQ03-20).

Declaration statement

No conflict of interest existed in the submission of this manuscript, and it was approved by all authors for publication.

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Table 1

The comprehensive index value, membership function value, value D and sorting result of 21 Chinese mustard cultivars (The abbreviation names of the 21 cultivars were specified in Table S2).

Cultivars	Comprehensive index value			Membership function value			Value D	Sorting
	CI-1	CI-2	CI-3	MF-1	MF-2	MF-3		
JTN	-0.778	-0.479	0.509	0	0.296	0.792	0.087	21
JSC	-0.576	-0.536	0.521	0.109	0.150	0.830	0.163	19
XXJ	-0.539	-0.482	0.482	0.129	0.288	0.711	0.186	18
ZX	-0.579	-0.572	0.430	0.107	0.057	0.552	0.132	20
BJ	1.073	-0.304	0.248	1.000	0.745	0.000	0.904	1
FJ-002	-0.091	-0.344	0.380	0.371	0.643	0.400	0.403	10
TJ-381	0.420	-0.204	0.341	0.647	1.000	0.282	0.662	3
KTJ	0.036	-0.313	0.464	0.440	0.721	0.655	0.486	8
JT-F1	0.217	-0.346	0.394	0.537	0.637	0.442	0.542	5
TJ-391	-0.408	-0.540	0.503	0.200	0.141	0.775	0.232	16
JXJ	-0.333	-0.391	0.420	0.240	0.520	0.521	0.291	15
CC-602	-0.272	-0.493	0.504	0.274	0.260	0.777	0.306	13
CC	-0.496	-0.595	0.549	0.152	0.000	0.913	0.187	17
BJ-3111	0.155	-0.347	0.358	0.504	0.634	0.334	0.507	6
BJ-338	-0.099	-0.471	0.435	0.367	0.318	0.568	0.375	11
DJ	0.072	-0.321	0.397	0.459	0.701	0.452	0.486	7
WJ	0.399	-0.273	0.430	0.636	0.822	0.554	0.651	4
HJ	0.569	-0.295	0.261	0.728	0.768	0.041	0.686	2
FJ-388	-0.338	-0.479	0.577	0.238	0.295	1.000	0.296	14
SJ	-0.142	-0.457	0.324	0.343	0.352	0.232	0.337	12
SY-16	0.012	-0.273	0.355	0.426	0.822	0.325	0.464	9

NOTE: The sum of the three membership function values multiplied by contribution rate of PC1 (principle component 1), PC2 (principle component 2) and PC3 (principle components 3) was defined as value D.

Figure captions:

Fig. 1. Tolerance indices of 21 Chinese mustard (*Brassica juncea* L.) cultivars under five Cd/Zn concentration exposure. The abscissa represents the 21 Chinese mustard (*Brassica juncea* L.) cultivars (the description of 21 cultivars was specified in Table S2). FX_{L1}, FX_{L2}, FX_{L3}, FX_{L4}, and FX_{L5} represent the soils with different Cd/Zn concentration for pot experiment. The total Cd contents of FX_{L1}–FX_{L5} were 1.14, 3.72, 6.65, 11.83 and 40.27 mg kg^{-1}, respectively. The total Zn contents of FX_{L1}–FX_{L5} were 325.9, 515.8, 862.5, 1274 and 3530 mg kg^{-1}, respectively.

Fig. 2. Multivariate analysis results. (A) is for principal component analysis. CI-1, CI-2 and CI-3 represent the three principle components, respectively. The black dots represent the 21 Chinese mustard (*Brassica juncea* L.) cultivars (the description of 21 cultivars was specified in Table S2). (B) is for cluster analysis of 21 Chinese mustard (*Brassica juncea* L.) cultivars. The ordinate represents the 21 Chinese mustard cultivars.

Fig. 3. Correlation between Cd/Zn content in soils (FX_{L0}, FX_{L1}, FX_{L2}, FX_{L3}, FX_{L4}, and FX_{L5}) and that in shoots and roots of BJ (Bao Jie, *var. involutus*) and JTN (Jiu Tou Niao, *var. multiceps*), respectively. (A) is for Cd content, (B) is for Zn content.

Fig. 4. Cd/Zn contents, AF and TF values of shoots and roots of 6 Chinese mustard cultivars in FX_{L5}. (A) is for Cd content, and (B) is for Zn content. AF: accumulation factor; TF: translocation factor. JTN, ZX, FJ-002, KTJ, HJ and BJ represent 6 Chinese mustard cultivars (Table S2). Data were presented as means ± SD.

Fig. 1.

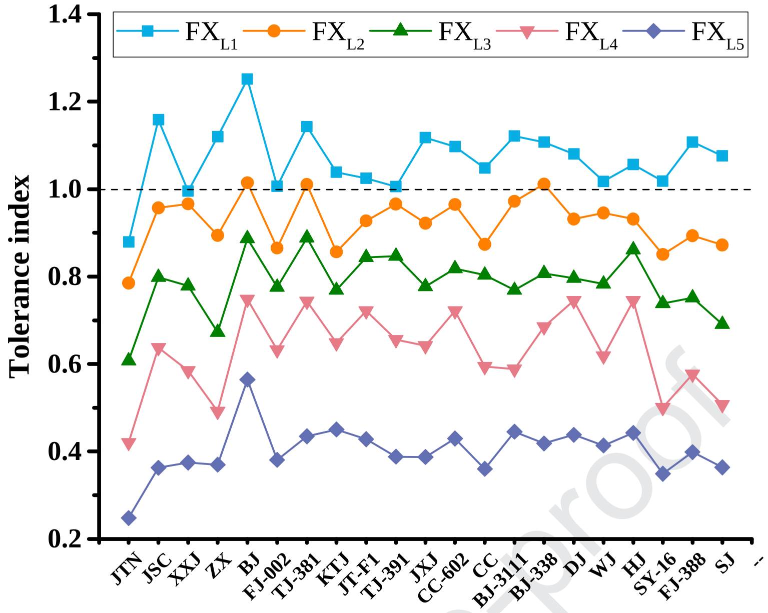
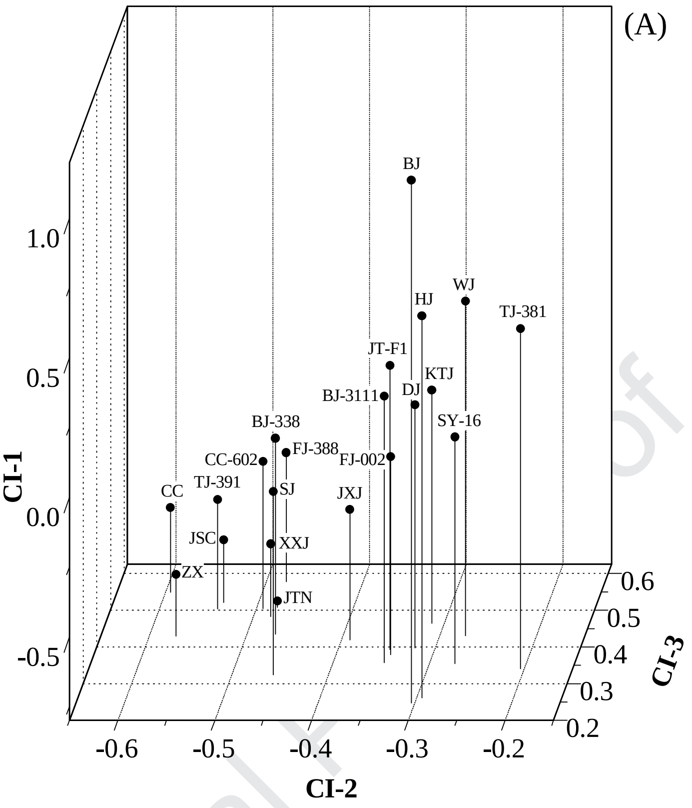

Tolerance index
1.4
1.2
1.0
0.8
0.6
0.4
0.2
FX_{L1}
FX_{L2}
FX_{L3}
FX_{L4}
FX_{L5}
JTN
JSC
XXJ
ZX
BJ
FJ-002
TJ-381
KTJ
JT-F1
TJ-391
JXJ
CC-602
CC
BJ-3111
BJ-338
DJ
WJ
HJ
SY-16
FJ-388
SJ

Fig. 2.


(A)
BJ
WJ
HJ
TJ-381
JT-F1
KTJ
BJ-3111
DJ
SY-16
BJ-338
FJ-388
CC-602
FJ-002
CC
TJ-391
SJ
JXJ
JSC
XXJ
ZX
JTN
CI-1
1.0
0.5
0.0
-0.5
-0.6
-0.5
-0.4
-0.3
-0.2
CI-2
0.6
0.5
0.4
0.3
0.2
CI-3

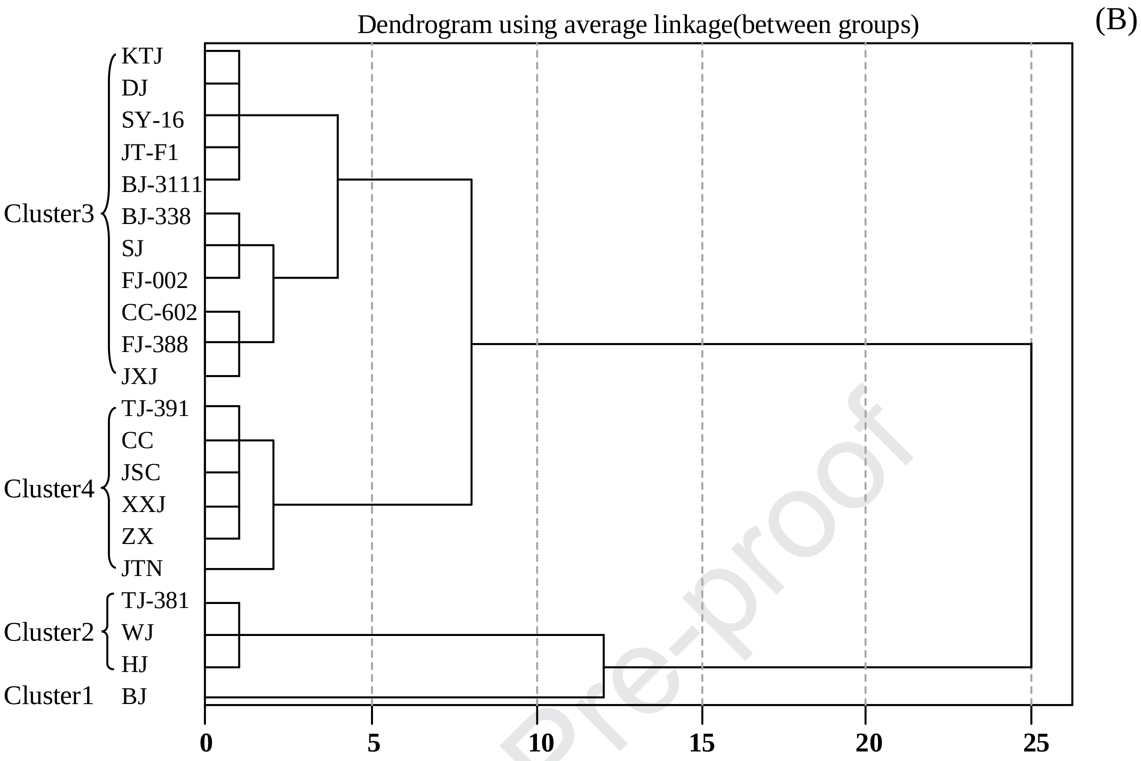

(B)
Dendrogram using average linkage(between groups)
Cluster3
KTJ
DJ
SY-16
JT-F1
BJ-3111
BJ-338
SJ
FJ-002
CC-602
FJ-388
JXJ
Cluster4
TJ-391
CC
JSC
XXJ
ZX
JTN
Cluster2
TJ-381
WJ
HJ
Cluster1
BJ
0
5
10
15
20
25

Fig. 3.

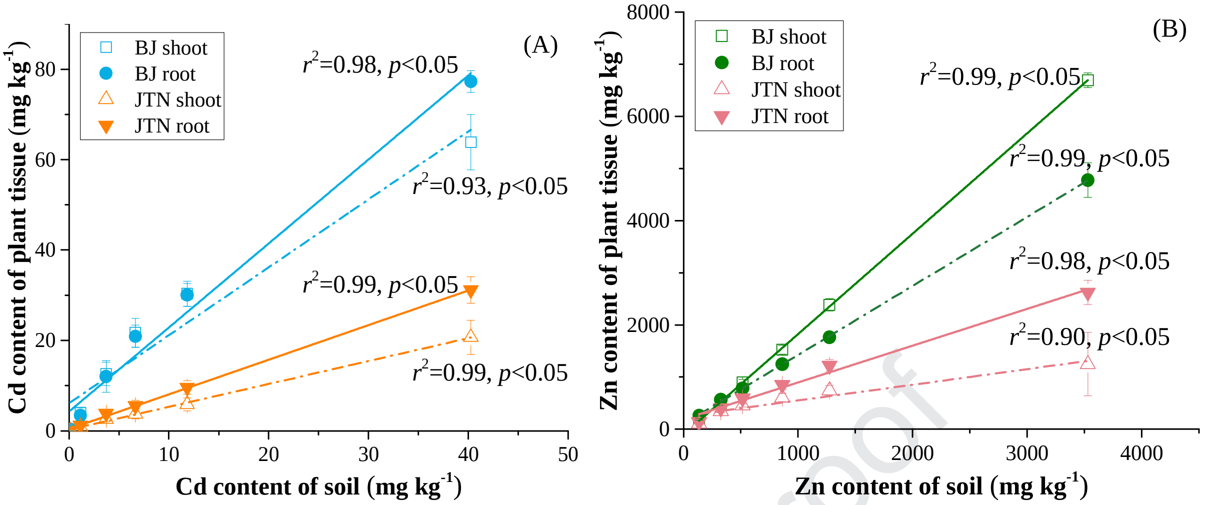
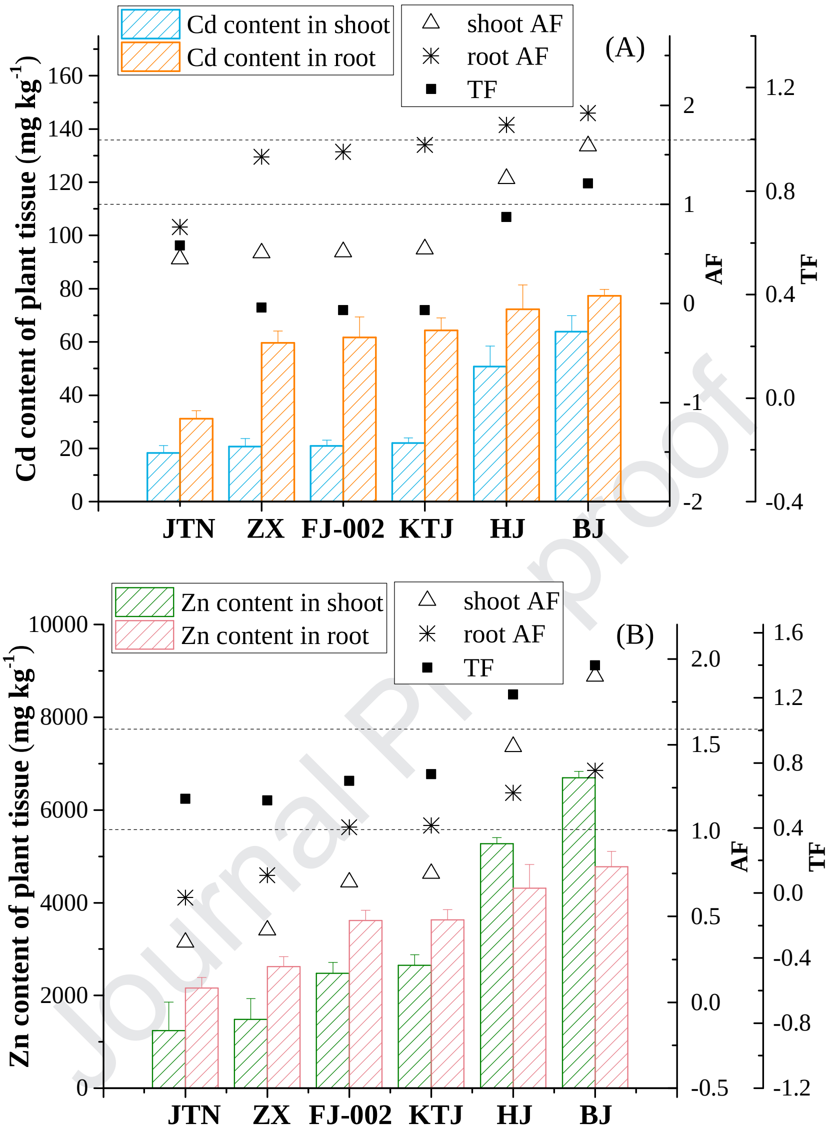

(A)
BJ shoot
BJ root
JTN shoot
JTN root
r^2=0.98, p<0.05
r^2=0.93, p<0.05
r^2=0.99, p<0.05
r^2=0.99, p<0.05
Cd content of plant tissue (mg kg^{-1})
Cd content of soil (mg kg^{-1})
0
20
40
60
80
0
10
20
30
40
50
(B)
BJ shoot
BJ root
JTN shoot
JTN root
r^2=0.99, p<0.05
r^2=0.99, p<0.05
r^2=0.98, p<0.05
r^2=0.90, p<0.05
Zn content of plant tissue (mg kg^{-1})
Zn content of soil (mg kg^{-1})
0
2000
4000
6000
8000
0
1000
2000
3000
4000

Fig. 4.


Cd content in shoot
Cd content in root
shoot AF
root AF
TF
(A)
Cd content of plant tissue (mg kg-1)
0
20
40
60
80
100
120
140
160
AF
-2
-1
0
1
2
TF
-0.4
0.0
0.4
0.8
1.2
JTN
ZX
FJ-002
KTJ
HJ
BJ
Zn content in shoot
Zn content in root
shoot AF
root AF
TF
(B)
Zn content of plant tissue (mg kg-1)
0
2000
4000
6000
8000
10000
AF
-0.5
0.0
0.5
1.0
1.5
2.0
TF
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
JTN
ZX
FJ-002
KTJ
HJ
BJ

Highlights:

- Chinese mustard cultivars for Cd/Zn phytoremediation were screened.
- BJ (Bao Jie, *var. involutus*) has the highest tolerance under Cd/Zn stress.
- BJ was identified as the most promising candidate for phytoremediation.
- The antioxidant system protected the plants from the injury of Cd/Zn stress.

We authors affirm the following statements:

The current manuscript has been submitted solely to this journal and it is not published, in press, or submitted elsewhere. All the research meets the ethical guidelines, including adherence to the legal requirements of the study country.

I have seen, read, and understood the journal’s guidelines on copyright. The names of all the co-authors have been included in the manuscript and these co-authors all had an active part in the final manuscript.

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Conflict of interest:

No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication.