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Subcellular Distribution of Metals within *Brassica chinensis* L. in Response to Elevated Lead and Chromium Stress

Zhipeng Wu,[†] Kim McGrouther,[§] Dongliang Chen,[#] Weidong Wu,*[,†] and Hailong Wang*[,†,⊥]

[†]Ministry of Education Key Laboratory of Protection and Development Utilization of Tropical Crop Germplasm Resources, Hainan University, Haikou 570228, China

[§]Scion, Private Bag 3020, Rotorua 3046, New Zealand

[#]Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100039, China

[⊥]School of Environmental and Resource Sciences, Zhejiang A & F University, Lin'an, Hangzhou 311300, China

ABSTRACT: Differential centrifugation and synchrotron radiation X-ray fluorescence spectroscopy (SRXRF) microprobe were used to study the distribution of the elements in tissue cross sections of pakchoi (*Brassica chinensis* L.) under stress of elevated Pb and Cr. Subcellular fractionation of the different tissues grown in a nutrient solution containing 200 mg L^{-1} Pb or 5 mg L^{-1} Cr showed that 86.7 and 76.3% of the Pb that accumulated in the roots and shoots, respectively, was contained in the cell wall and vacuoles in those areas. Whereas 75.0% of the Cr that accumulated in the root was contained in the cell wall, 63.1% of the Cr that accumulated in the shoot was found in the vacuoles and cell wall. SRXRF analysis revealed that, when pakchoi seedlings were placed under excess Pb stress, the Pb, Ca, Cu, and Zn were concentrated in the cortex and vascular bundle of the root and mixed Fe−Mn plaques were seen on the surface of the pakchoi root. In the Cr treatment group, Cr, Ca, Mn, and Zn were mainly located in the cortex of the root, whereas in the stem, only Ca, Cu, and Zn were detected at higher levels in the cortex area. Thus, this study provides evidence that, in response to Pb and Cr stress, pakchoi uses cell walls and vacuoles to reduce the transport of these heavy metals through the plant, as well as restrict transport from the root to the stem.

KEYWORDS: *Brassica chinensis, differential centrifugation, heavy metals, subcellular distribution, pakchoi, SRXRF, synchrotron*

INTRODUCTION

High concentrations of chromium (Cr) and lead (Pb) can impair plant photosynthesis, respiration, and nitrogen assimilation, alter mineral nutrition balance, and stunt plant growth and development.[1,2] These heavy metals could be transferred to the food chain and threaten human and animal health. Pb and Cr are mainly released from the mining, leather, and metallurgical industries.[1,3]

However, plants grown in heavy metal-contaminated soils have developed specialized mechanisms, which vary from species to species, in response to heavy metal stress.[4] A plant's resistance or tolerance to heavy metals can be classified into two basic strategies. The first strategy is to restrict the uptake and accumulation of metals by preventing metal transport across the plasma membrane via modified ion channels; increasing binding of metal ions to the cell wall; or taking the metal out of the cell with active efflux pumps.[5−7] The second strategy is to detoxify heavy metal ions entering the plants through compartmentalization via accumulation in different plant tissues or cell organelles and/or inactivation via chelation or conversion to a nontoxic ion form.[8] For example, MacFarlane and Burchett[9] found that the casparian strip and endodermis may resist Cu, Pb, and Zn entry into the root stele, thus restricting uptake into the plant. Whereas Zeng et al.[10] showed evidence of chromium compartmentalization in rice, Cr in the roots was mainly localized in cell walls, and Cr was restricted to the vacuoles and cell walls in the leaves and stems. Other researchers have shown that toxic metal movement in some plants was restricted due to chelation by metallothioneins (MTs) and phytochelatins (PCs).[11,12] Hence, localization of heavy metals in plant tissues and subcellular distribution is closely involved in their toxicity and tolerance[13] and can provide essential information on metal toxicity and tolerance mechanisms.[14] To date, most of the research has focused on the subcellular localization of heavy metals such as Cd, Zn, Ni, and Cu; little is known regarding Pb or Cr accumulation and localization.

Besides the compartmentalization of toxic elements, determination of the distribution of elements in heavy metal stressed plants could make a significant contribution to understanding the detoxification mechanisms of plants.[15] Energetic beam characterization techniques such as energy-dispersive X-ray microanalysis (EDXA), transmission electron microscope (TEM), electron spectroscopic imaging (ESI), electron energy loss spectroscopy (EELS), and proton-induced X-ray analysis (PIXE) have been used to study toxic metal localization and tolerance mechanisms in plants.[16] Compared with the other techniques, synchrotron radiation X-ray fluorescence spectroscopy (SRXRF) microprobe is more sensitive and results in less radiation damage to cells. SRXRF is also compatible with fully hydrated biological samples such as whole cells, tissue sections, or the whole living plant while simultaneously offering trace element sensitivity and submi-

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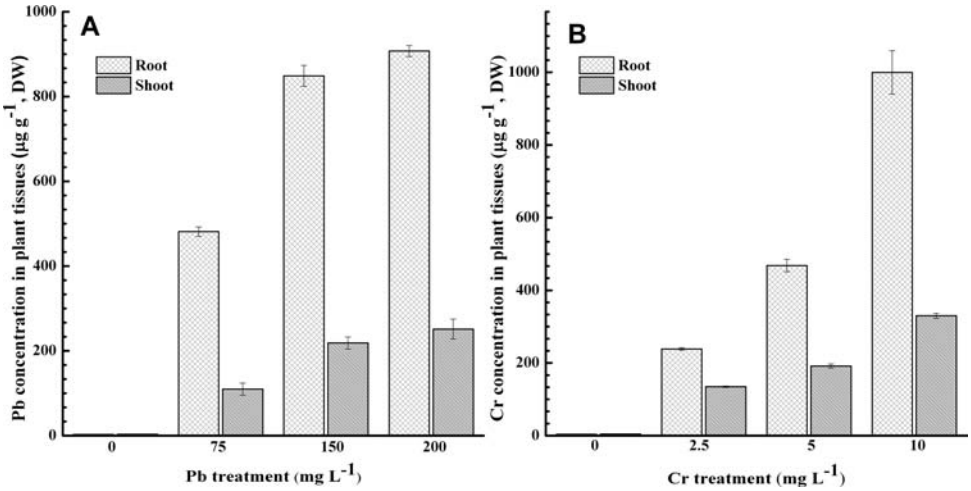

Figure 1. Pb (A) and Cr (B) concentrations (μg g^{-1} DW) in the roots and shoots of pakchoi (*Brassica chinensis* L.) (n = 3). DW = dry weight.

crometer spatial resolution.[17,18] Yun et al.[19] first applied X-ray fluorescence (XRF) imaging and X-ray absorption spectroscopy (XAS) from synchrotron radiation at the Argonne National Laboratory (USA) in a study to determine the elemental distribution in mycorrhizal plant roots and fungi in their natural hydrated state. Since then, SRXRF has been used to determine the distribution of metals such as Cu, Zn, and Pb, in various tissues of plants.[20−22]

Suzhouqing pakchoi (*Brassica chinensis* L.) is one of the *Brassica* species that shows an ability to absorb and accumulate high levels of heavy metals.[23] Suzhouqing pakchoi is also a widely cultivated leafy vegetable in China. The aims of this study were to investigate (a) tissue and subcellular distribution of toxic metals (Pb, Cr) in the shoot and root of Suzhouquig pakchoi by ICP-MS and differential centrifugation and (b) the distribution of Cr, Pb, Ca, Mn, Fe, Cu, and Zn in cross sections of the root and stem of Suzhouqing pakchoi by SRXRF.

MATERIALS AND METHODS

Materials. Suzhouqing pakchoi (*B. chinensis* L.) seeds from Yonghui Seed Co. in Hebei province were used in this experiment. A modified Hoagland nutrient solution (pH 6.5) was prepared with the following macronutrients (1.25 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.25 mM KH_2PO_4, and 1.25 mM KNO_3) and micronutrients (4.5 μM $MnCl_2{\cdot}4H_2O$, 10 μM Fe(III) citrate, 0.19 μM $ZnSO_4{\cdot}7H_2O$, 11.6 μM H_3BO_3, 0.12 μM $Na_2MoO_4{\cdot}2H_2O$, and 0.08 μM $CuSO_4{\cdot}5H_2O$). The sand was obtained from an uncontaminated river bank and washed with deionized water prior to use.

Plant Culture. The seeds (10 g) were washed with tap water for 30 min and rinsed twice with ultrapure water (resistivity = 18.2 MΩ). The seeds were then soaked in ultrapure water (300 mL) in the dark for 24 h at 25 ± 1 °C and germinated on moistened cotton cloth in a porcelain dish at 27 ± 1 °C in a standing-temperature cultivator for 2 days. Afterward, the seedlings were transferred into a sand container and placed in a controlled environment with a photoperiod of 8 h (20 °C) dark/16 h (25 °C) light at 75% relative humidity. The modified Hoagland nutrient solution (300 mL) was applied to the sand container prior to addition of the seedlings. After 2 weeks, the seedlings were transplanted into separate sand containers, which were soaked in heavy metal-contaminated nutrient solution containing $Pb(NO_3)_2$ and $CrCl_3$, forming three levels of Pb (0, 75, 150, 200 mg L^{-1}) and Cr (0, 2.5, 5, 10 mg L^{-1}), respectively. The experiment was set up in a completely randomized design with three replicates per treatment. The plants were harvested for analysis after 4 weeks of heavy metal exposure. During the whole experiment, the sand was rinsed with ultrapure water, and then the fresh nutrient solution was renewed three times a week.

Separation and ICP-MS Measurement of Subcellular Fractions. Fresh plants were washed with ultrapure water (500 mL) and divided into roots and shoots. The subcellular fractions for heavy metal analysis were prepared according to the method of Zeng et al.[10] with some modification. The roots and shoots were weighed and homogenized in a medium containing 250 mM sucrose, 50 mM Tris-HCl (pH 7.5), and 1.0 mM dithioerythritol (DTE), which had been cooled to 4 °C prior to addition of the homogenate. The homogenate was filtered through a nylon cloth (80 μm), and the residue on the nylon cloth was considered to be the cell-wall fraction (FI). The filtrate was centrifuged at 2500g for 20 min (shoot sample 1500g, 10 min) at 4 °C using a refrigerated centrifuge (Thermo Scientific, Sorvall ST 16R). The pellet at the bottom of centrifugal tube was designated plastids (FII). The supernatant of the first centrifugation step was then centrifuged at 5000g for 20 min, and the remaining pellet was named as the nucleus fraction (FIII). Then the supernatant was further centrifuged at 5000g for 20 min to separate the cell sediment, which was classified as the mitochondrial fraction (FIV). The last supernatant was designated the soluble fraction (FV). All subcellular fractions of roots and shoots were freeze-dried and then digested with concentrated acid mixture of HNO_3 and H_2O_2 (3 mL; 2:1 v/v) per 0.3 g of plant material in a microwave digestion system (SINED, MDS-6). Metal (Pb, Cr) concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS; Thermo Fisher-X series).

Sample Preparation and SRXRF Analysis. Roots and shoots for SRXRF analysis were gently washed with ultrapure water (200 mL) and quickly frozen in liquid nitrogen (−167 °C) for 30 min. Samples were taken from the liquid nitrogen and were completely immersed in water, in aluminum foil containers, before freezing at −20 °C. The aim of complete immersion was to reduce sample damage during cutting. Afterward, cross sections (50 μm) of root (meristematic zone) and stem were cut with a cryomicrotome (Leica CM1950) at −20 °C and subsequently freeze-dried for 48 h. Samples were stored at −80 °C for further analysis.

Cross-section scanning was performed by micro-XRF at the XRF station in the Beijing Synchrotron Radiation Facility, Beijing, China. The X-ray light was multichromatic light (white light) provided by a 4W1B beamline station, with an energy range from 5 to 18.5 keV. The electron storage ring was 2.2 GeV with a current range from 67 to 150 mA. The distance between the XRF microprobe experimental station and the point of the light source was 25 m. The emission angles of the light in horizontal and vertical directions were 1.0 and 0.1 mrad, respectively. The XRF spectra were detected using a PGT Si (Li) solid detector. During the micro-XRF experiments, the spot size of the X-ray was fixed at 30 × 30 μm^2. The scanning dot moved according to the displacement ΔX = 50 μm, ΔY = 50 μm, and each dot had been

Table 1. Lead and Chromium Concentration in Subcellular Fractions from the Roots and Shoots of Pakchoi Plants under Different Pb and Cr Levels (n = 3)[a]

heavy metal, mg L^{-1}		metal concentrations, μg g^{-1} DW					
		FI	FII	FIII	FIV	FV	total
				Roots			
Pb	0	0.730 ± 0.04d	0.330 ± 0.04d	0.320 ± 0.02c	0.620 ± 0.03c	0.640 ± 0.03c	2.65 ± 0.16d
	75	194 ± 8.24c	52.5 ± 2.03c	73.2 ± 1.35a	38.5 ± 0.98b	123 ± 1.89b	481 ± 10.8c
	150	463 ± 6.44b	78.1 ± 2.75a	11.9 ± 0.97b	37.2 ± 1.42b	256 ± 3.31a	849 ± 24.9b
	200	544 ± 7.22a	65.3 ± 2.04b	9.97 ± 0.46b	45.8 ± 1.31a	250 ± 3.24a	907 ± 13.1a
Cr	0	0.940 ± 0.06d	0.610 ± 0.03c	0.49 ± 0.03d	0.760 ± 0.02c	1.01 ± 0.04d	3.83 ± 0.19d
	2.5	131 ± 4.15c	52.8 ± 2.14b	7.64 ± 0.27b	10.0 ± 0.12b	37.7 ± 0.79c	239 ± 3.38c
	5	351 ± 6.09b	47.3 ± 1.42b	5.61 ± 0.24c	9.83 ± 0.22b	54.3 ± 11.42b	468 ± 17.4b
	10	710 ± 8.94a	76.0 ± 3.69a	17.9 ± 0.32a	37.9 ± 0.32a	167.1 ± 5.39a	1000 ± 59.8a
				Shoots			
Pb	0	0.57 ± 0.04d	0.680 ± 0.03c	0.530 ± 0.02d	0.700 ± 0.03c	0.690 ± 0.04d	3.18 ± 0.09c
	75	27.0 ± 1.04c	18.9 ± 1.42b	16.8 ± 0.55b	17.9 ± 0.46c	40.0 ± 2.01c	110 ± 14.12b
	150	45.9 ± 0.73b	27.54 ± 0.78a	20.6 ± 0.73a	31.6 ± 1.54a	93.6 ± 2.02b	219 ± 14.38a
	200	56.0 ± 0.72a	25.4 ± 1.12a	8.54 ± 0.39c	25.6 ± 1.39b	136 ± 2.51a	251 ± 23.39a
Cr	0	0.860 ± 0.06d	0.980 ± 0.07d	0.800 ± 0.04c	0.910 ± 0.05d	0.890 ± 0.06d	4.45 ± 0.20d
	2.5	38.9 ± 1.38c	24.2 ± 1.47c	20.1 ± 0.49b	16.6 ± 0.40c	35.4 ± 0.85c	135 ± 1.84c
	5	48.1 ± 2.23b	28.2 ± 0.86b	18.6 ± 0.32b	24.0 ± 0.77b	72.8 ± 1.31b	192 ± 5.82b
	10	99.0 ± 2.67a	39.6 ± 1.50a	33.0 ± 1.23a	49.8 ± 1.79a	122 ± 2.93a	330 ± 6.96a

[a]Results are means ± SD (n = 3). Values followed by different lower case letters within the same line are significantly different between Pb (Cr) treatments at 5% level (p < 0.05) according to Tukey's multiple-range test. DW = dry weight. Cells were separated into different fractions: cell wall (FI), plastid (FII), nucleus (FIII), mitochondrion (FIV), supernatant soluble fraction (FV) by gradient centrifugation technique at 4 °C.

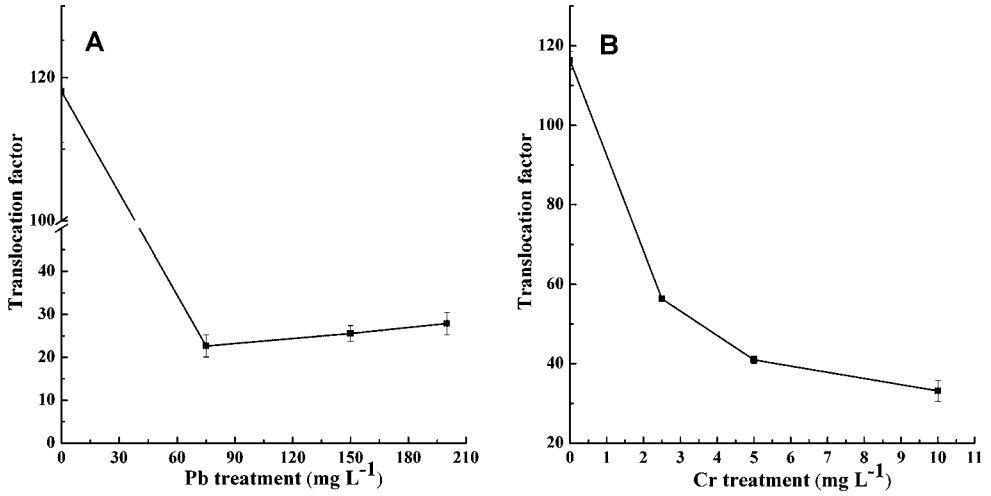

Figure 2. Translocation factors of Pb (A) and Cr (B) from the root to shoot of pakchoi (*B. chinensis* L.) (n = 3).

scanned for 20 s. Details of SRXRF scanning apparatus were reported by Huang et al.[24] The XRF spectra were analyzed using PyMCA software from ESRF and peak areas calculated after background correction.

Statistical Analysis. Statistical analysis was performed using software packages SAS 9.1 and Origin 8.6. All data values are described in the form of mean ± SD. All variables were analyzed by one-way ANOVA (p < 0.05) according to Tukey's multiple-range tests (with a 95.0% confidence level). Multielemental correlation analysis and cluster analysis were performed. The translocation factor (TF) was calculated from the tissue concentrations toxic metals (Pb and Cr) to evaluate the plant's ability to translocate heavy metals from the roots to the shoots:[25] TF = 100 × C_{shoot}/C_{root}, where C = concentration of metal in mg kg^{-1} and shoot = stem and leaves.

RESULTS

Tissue Distribution of Pb and Cr. Lead (Pb) and chromium (Cr) concentrations in different root and shoot tissues from pakchoi are shown in Figure 1. Pb and Cr in each tissue increased significantly (p < 0.05) with increasing Pb and Cr levels in the culture solution (Table 1). The Pb and Cr maximum concentrations of 907 and 1000 μg g^{-1}, respectively, were detected in the roots. The Pb and Cr maximum concentrations in the shoots were 251 and 330 μg g^{-1}, respectively. Pb and Cr concentrations in the roots were significantly higher than those detected in the shoots. The translocation factors (22.9−27.7) from the root to the shoot increased as the applied Pb concentration increased from 75 to 200 mg L^{-1} (Figure 2A). The Cr translocation factors (56.5−33.0) decreased as the applied Cr concentrations increased from 2.5 to 10 mg L^{-1} (Figure 2B). The above results suggest that Pb and Cr were mainly stored in roots of Suzhouqing pakchoi (*B. chinensis* L.) in response to heavy metal stress but that the resistance to transfer was lower for Pb than it was for Cr.

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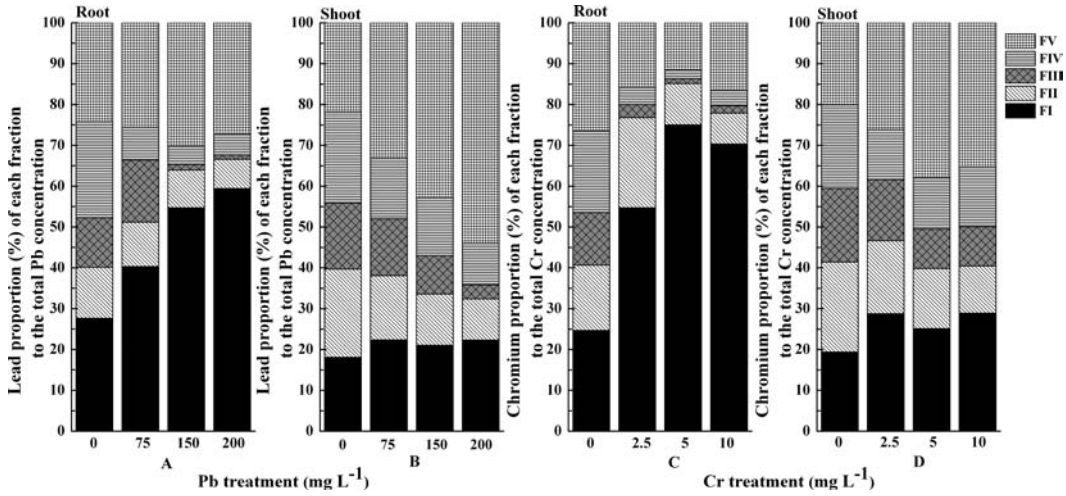

Figure 3. Subcellular proportions of Pb (A, B) and Cr (C, D) in roots and shoots of pakchoi plants under different Pb and Cr levels; cell wall (FI), plastid (FII), nucleus (FIII), mitochondrion (FIV), supernatant soluble fraction (FV).

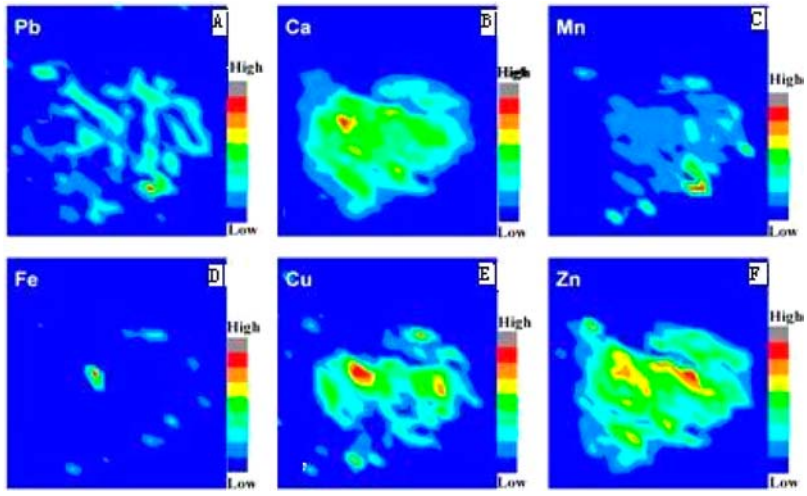

Figure 4. SRXRF images of Pb, Ca, Mn, Fe, Cu, and Zn distribution in cross sections of pakchoi root in response to 150 mg L^{-1} Pb. Higher fluorescence intensities (corresponding to higher concentrations) are nearer to red and lower intensities are nearer to blue according to the color bars.

Subcellular Distribution of Pb and Cr. Pb and Cr concentrations in the subcellular fractions from roots and shoots of Suzhouqing pakchoi (*B. chinensis* L.) are listed in Table 1. Overall, the Pb concentration in the different subcellular fractions (except for FIII, nucleus) increased with increasing Pb level in the medium. For instance, compared with the plants exposed to 75 mg L^{-1} Pb, the Pb concentrations in the cell wall (FI), plastid (FII), mitochondria (FIV), and supernatant soluble fraction (FV) of the plants exposed to 200 mg L^{-1} Pb increased 2.81-, 1.25-, 1.19-, and 2.04-fold in roots and 2.08-, 1.34-, 1.43-, and 3.39-fold in shoots, whereas the Pb concentration in the nucleus (FIII) reduced by 86.4% in roots and 49.1% in shoots, respectively. In all cases, the Cr concentration in each subcellular fraction rose with the addition of Cr in the medium. As the Cr concentration in the medium changed from 2.5 to 10 mg L^{-1}, the Cr concentrations in cell wall (FI), plastid (FII), nucleus (FIII), mitochondria (FIV), and supernatant soluble fraction (FV) of the plants increased, respectively, 5.43-, 1.44-, 2.35-, 3.79-, and 4.43-fold in roots and 2.55-, 1.63-, 1.64-, 3.01-, and 3.43-fold in shoots.

The distribution of heavy metals between various subcellular fractions of the roots and shoots were different for Pb and Cr (Figure 3). The Pb concentrations followed the orders FI > FV > FIV > FII > FIII (in roots) and FV > FI > FIV > FII > FIII (in shoots) (Figure 3A,B), whereas the Cr proportion in different subcellular fractions had the following the orders: FI > FV > FII > FIV > FIII (in roots) and FV > F I > FII > FIV > FIII (in shoots) (Figure 3C,D). On average, across the four Pb levels applied, the cell wall fraction (FI) of the roots accounted for the largest accumulation of Pb (42.7%), followed by the supernatant soluble fraction (FV) at 26.9% (Figure 3A,B). The supernatant soluble fraction (FV) represented the largest share of 38.0% of the total Pb in shoots, which was followed by the cell wall fraction (FI) Pb (20.9%). Similarly, the proportion of Cr was largest in the cell wall (FI) (Figure 3C,D) of the roots (56.2%) and in the supernatant soluble fraction of the shoots (29.9%).

Moreover, the Pb and Cr proportion of each subcellular fraction varied with the Pb and Cr level in the culture solution (Figure 3). As the Pb level increased from 75 to 200 mg L^{-1}, the Pb proportion in cell wall (FI) of roots (Figure 3A)

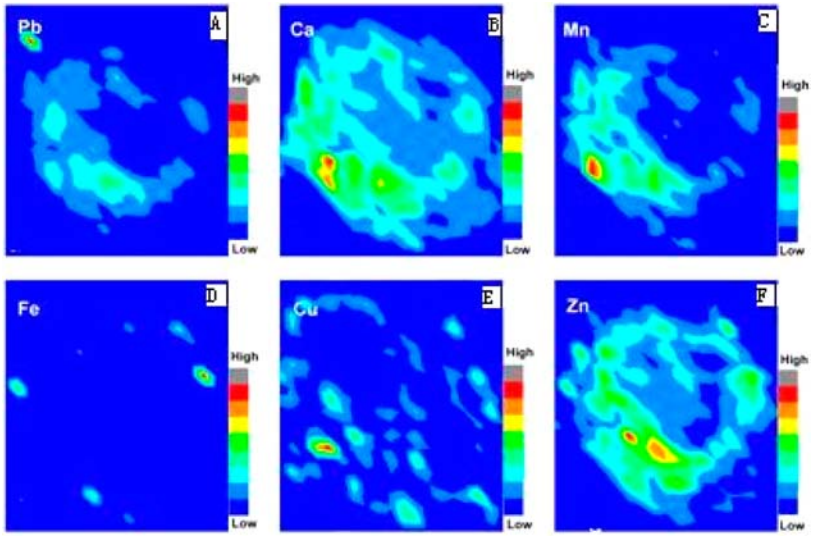

Figure 5. SRXRF images of Pb, Ca, Mn, Fe, Cu, and Zn distribution in cross sections of pakchoi stem in response to 150 mg L^{-1} Pb. Higher fluorescence intensities (corresponding to higher concentrations) are nearer to red and lower intensities are nearer to blue according to the color bars.

Table 2. Pearson Correlation Coefficients between Pb and Cr and Other Elements in the Cross Section of Root and Stem of Pakchoi Plants[a]

	Ca	Mn	Fe	Cu	Zn
			Pb		
root (n = 504)	0.478**	0.663**	0.325**	0.444**	0.547**
stem (n = 418)	0.756**	0.759**	0.094^{-}	0.13**	0.758**
			Cr		
root (n = 342)	0.752**	0.613**	0.574**	0.427**	0.667**
stem (n = 550)	0.295**	0.421**	0.645**	0.116**	0.313**

[a]Two-tailed test of significance was used: ** means the correlation is significant at the 0.01 level; "$^{-}$" means the correlation is not significant at the 0.05 or 0.01 level. "n" indicates the number of scanning points.

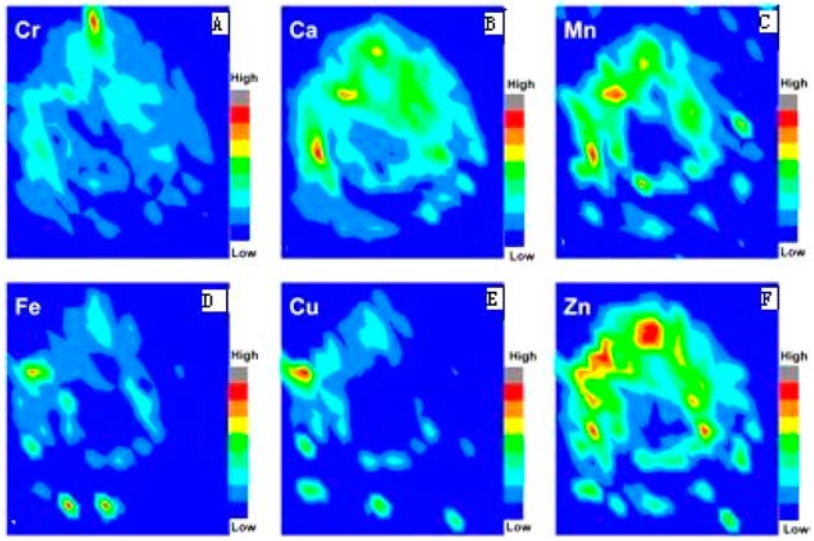

Figure 6. SRXRF images of Cr, Ca, Mn, Fe, Cu, and Zn distribution in cross sections of pakchoi root in response to 10 mg L^{-1} Cr. Higher fluorescence intensities (corresponding to higher concentrations) are nearer to red and lower intensities are nearer to blue according to the color bars.

increased from 29.0 to 59.4%. In contrast, the supernatant soluble fraction (FV) displayed little change (25.6−27.3%), and the Pb proportion in the remaining subcellular fractions (FII, FIII, FIV) was significantly diminished. The Pb proportion in supernatant soluble fraction (FV) in shoots (Figure 3B) increased up to 54.0% as the feed concentration increased from 75 to 200 mg L^{-1} Pb, whereas the nucleus fraction (FIII) decreased. As for Cr (Figure 3C,D), >70.0% of the root Cr was

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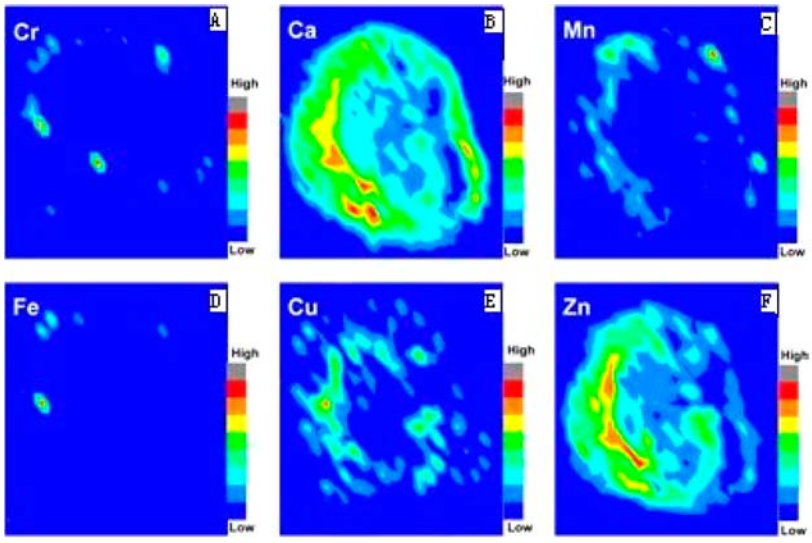

Figure 7. SRXRF images of Cr, Ca, Mn, Fe, Cu, and Zn distribution in cross sections of pakchoi stem in response to 10 mg L^{-1} Cr. Higher fluorescence intensities (corresponding to higher concentrations) are nearer to red and lower intensities are nearer to blue according to the color bars.

sequestered predominantly in the FI fraction (cell wall) for the plant exposed to 10 mg L^{-1} Cr, but little Cr was found in other fractions. In plant shoots, the largest proportion of Cr (38.0%) was observed in the FV fraction from the plant treated with the 5 mg L^{-1} level of Cr, and the Cr proportion in FI fraction accounted for over a fourth (25.1−28.9%) of the total Cr amount.

Spatial Distribution of Pb and Mineral Elements in Cross Sections of Roots and Stems. The SRXRF scanning maps of cross sections of roots and stems, subjected to 150 mg L^{-1}Pb, are shown in Figures 4 and 5. The Pb intensities in the alveolate profile of the whole cross section of the root show that Pb distribution is mainly in the apoplastic (cell wall, intercellular space) structure, which correlates with what was seen using ICP-MS. The lowest intensities of Ca, Cu, and Zn were observed in the rhizodermis, and hot points of their intensities were detected in the cortex and endoderm (Figure 4B,E,F). In contrast, hot points of Fe and Mn intensities were mainly found in the rhizodermis of the root (Figure 4C,D). According to the Pearson correlation coefficient (Table 2), Pb was significantly related with the other elements (largest coefficient, Pb−Mn, 0.663**; smallest coefficient, Pb−Fe, 0.325**). As for the cross section of the stem, the lowest intensities of Ca, Mn, Cu, Zn, and Pb were seen in the central pith, and hot points were mostly visible in the cortex and vascular bundle at the left of the cross section of the stem (Figure 5B,C,F). The intensities of Fe were sporadically distributed in the epidermis (Figure 5D,E). The distributions of Ca, Mn, Zn, and Pb were significantly correlated, whereas Pb was not associated with the distribution of Fe (Table 2).

Spatial Distribution of Cr and Mineral Elements in Cross Sections of Roots and Stems. For the plants subjected to 10 mg L^{-1} chromium stress, different elemental distribution patterns in the whole cross section of root and stem were found in the SRXRF maps (Figures 6 and 7). In general, the most visible intensities of all elements were detected in the upper half of the cross section of the root. The highest contents of Cr, Ca, Mn, and Zn were accumulated in the cortex of the root, and the lowest content was accumulated in the vascular bundle (Figure 6A−C,F). In contrast, the highest Fe and Cu concentrations were observed in the rhizodermis and endoderm (Figure 6D,E). As shown in Table 2, the distribution of Cr and other mineral elements (e.g., Ca, Mn, Fe, and Zn) was significantly related.

In the cross section of stem, the highest Ca, Cu, and Zn contents were found in the cortex area (Figure 7B,E,F), whereas the visible intensities of Cr, Mn, and Fe were mainly detected in the epidermis (Figure 7A,C,D) and were related to the intensities of Cr (Table 2: Cr−Mn, 0.421**; Cr−Fe, 0.645**).

DISCUSSION

Heavy metal ions are taken up primarily through the roots and retained there, whereas their translocation to aboveground parts is restricted.[26] Heavy metal ion accumulation in the roots is a very important tolerance mechanism to metal stress for plants. It is evident from the current results that the Pb and Cr contents in *B. chinensis* L. pakchoi roots exposed to the highest metal levels are approximately 3.66 and 3.02 times that in corresponding plant shoots, respectively (Figure 1). The Pb and Cr translocation factors from roots to shoots gradually increases with the addition of Pb level (except the control), whereas that of Cr decreases as the applied metal concentration increases (Figure 2A,B). Cenkci et al.[27] reported a similar Pb translocation trend when they investigated heavy metal stress in *Brassica rapa* L.;[28] the Pb concentration in the shoots (stem, old leaf, young leaf, head) of cabbage (*Brassica oleracea* L.) increased with an increase in Pb supply when compared to the roots. Zayed et al.[29] have observed in research on 11 vegetable plant species that translocation of both Cr forms (Cr^{3+}, CrO_4^{2-}) from the roots to shoots was extremely limited and that the highest Cr concentrations were found in members of the Brassicaceae family such as cabbage, cauliflower, and kale. It can be concluded that binding metals (i.e., Cr and Pb) in the root tissues is a crucial tolerant strategy in Suzhouqing pakchoi.

The cell wall is considered to be the first barrier of heavy metal entrance into cells.[30] The cell wall's ability to bind divalent and trivalent metal cations mainly depends on the amount of carboxylic-rich polysaccharides.[31] Excessive levels of toxic metal cations can enter the plant cell through the plasma

membrane and are mostly sequestered by vacuoles within the cells to protect the cell contents from the toxicity of metals.[32] Therefore, the cell wall and vacuoles act as two key storage compartment fractions of metals in plants.[33] Our study results show that Pb in the pakchoi is predominantly accumulated in the cell wall and vacuoles of the root (average proportions: 45.5 and 26.9%, respectively) and shoot (average proportions: 20.9 and 38.0%, respectively) (Figure 3A,B). Another heavy metal uptake study showed a similar Pb uptake mechanism in signal grass, where Pb was mainly accumulated in the apoplast (cell wall, intercellular space) of the roots.[20] Peng et al.[34] found that about 50.0% of the Pb in the leaves of *Ptamogeton pectinatus* accumulated in cell wall (about 25.0%) and vacuoles (about 25.0%). In contrast to Pb, our results show that Cr is mostly localized in cell wall (>70%) of the root (Figure 3C). The Cr that is transferred to the shoot is stored, in a similar manner to that seen with Pb, in the vacuoles and cell wall (on average, 29.9 and 25.5%, respectively) (Figure 3D). Zeng et al.[10] also found, with rice, that the highest concentration of Cr occurred in the cell-wall fraction of roots and in the supernatant soluble fraction of leaves. The highest Cr concentration in wheat and bean leaves was found in the supernatant soluble fraction (vacuolar sap and ribosomes).[35] Moreover, in the current study, the Pb concentration in the nucleus fraction of the root and the shoot significantly decreased from the 75−200 mg L^{-1} level (Figure 3A,B), indicating that the plant may be actively restricting the level of Pb in the nucleus as a protective mechanism.

Endodermal and exodermal/hypodermal cell walls should form special apoplastic barriers for toxic metals radially diffusing from the solution to the xylem vessels in roots.[36] Our results indicate that Pb is mainly distributed along apoplastic structures, cell wall and intercellular space, on the whole cross section by SRXRF (Figure 4A). A study on the root of *Raphanus sativas*[37] demonstrated that Pb was sequestered in eipidermis and endodermis, whereas a small amount could be detected in the vascular tissues. Cr was mainly accumulated in the cortex of the root in the current study (Figure 6A). Caldelas and Bort[38] found that the Cr concentration in root tissues of *Iris pseudacorus* L. was in the order cortex > rhizodermis > stele, whereas Mangabeira et al.[39] analyzed tomato roots by ion microscopy and found that Cr accumulated preferentially in the vascular tissues. Pb and Cr were mainly contained in the epidermis and vascular areas of the roots in our study (Figures 4A and 6A). Clemens[40] noted that metal-hydrated ions or metal−chelate complexes inside the xylem can restrict metals' availability to move from the root to the shoot.

Moreover, the scanning maps of cross sections of the root and shoot by SRXRF demonstrate that these elements are more predominantly carried into the root tissue and only mainly deposited in the xylem or adsorbed in or on the rhizodermis (Figures 4−7). The difference in the distribution between Fe and other elements can be explained by their chemical properties. Fe is a redox-sensitive element and can accumulate on oxidation at the root surface. Previously, Fe plaques have been reported for other plants such as *Oryza sativa* L.[41] and *Commelina communis*.[20] However, in this study, the plaques observed on the surface of the root in the Pb treatment group also contained Mn. The involvement of Mn in these plaques may be due to Mn maintaining lower redox potential at the root surface in the permeable sand culture medium.[42] The metal elements (e.g., Pb, Ca, Cu, and Zn) can be complexed by organic acids for translocation in plants or are precipitated in the plants by chloropyromorphite or oxalate or pectin.[22,43,44]

The SRXRF technique proved to be an ideal technique for our study of metal localization in plants as there was limited sample pretreatment, no destruction to samples, and rapid simultaneous measurement of multiple elements.[45] SRXRF also has a high detection sensitivity with elemental abundances in the microgram per gram range.[46]

In conclusion, Suzhouqing pakchoi (*B. chinensis* L.) appears to have a strong tolerance and accumulation capacity for Pb and Cr as evidenced by the accumulation and distribution of Pb and Cr in tissue and subcellular spaces in the roots and shoots. Most of the Pb and Cr was accumulated in the roots, and the translocation of Pb from roots to shoots was gradually enhanced with the increasing Pb concentrations (except for the control), whereas that of Cr was diminished. By differential centrifugation technology, pakchoi's tolerance to Pb in the root and shoot was mainly attributed to both the cell wall and vacuoles. Chromium was mainly accumulated in the cell wall of the root and in both vacuoles and cell wall of the shoot. The distributions of both Pb and Cr in the root were significantly related to the other elements. In the stem, the distribution of Pb was not correlated with Fe, and the distribution of Cr was not correlated with Cu. In the Pb group, Fe−Mn plaques were detected on the pakchoi root surface.

AUTHOR INFORMATION

Corresponding Author

*E-mail: wdwu@hainu.edu.cn (W.W.); nzhailongwang@gmail.com (H.W.) Phone: +86 898 6625 1724. Fax: +86 898 6625 1724.

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