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High Accumulation and Subcellular Distribution of Thallium in Green Cabbage (*Brassica Oleracea* L. *Var. Capitata* L.)

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The accumulation of thallium (Tl) in brassicaceous crops is widely known, but both the uptake extents of Tl by the individual cultivars of green cabbage and the distribution of Tl in the tissues of green cabbage are not well understood. Five commonly available cultivars of green cabbage grown in the Tl-spiked pot-culture trials were studied for the uptake extent and subcellular distribution of Tl. The results showed that all the trial cultivars mainly concentrated Tl in the leaves (101∼192 mg/kg, DW) rather than in the roots or stems, with no significant differences among cultivars ($p = 0.455$). Tl accumulation in the leaves revealed obvious subcellular fractionation: cell cytosol and vacuole >> cell wall > cell organelles. The majority (∼ 88%) of leaf-Tl was found to be in the fraction of cytosol and vacuole, which also served as the major storage site for other major elements such as Ca and Mg. This specific subcellular fractionation of Tl appeared to enable green cabbage to avoid Tl damage to its vital organelles and to help green cabbage tolerate and detoxify Tl. This study demonstrated that all the five green cabbage cultivars show a good application potential in the phytoremediation of Tl-contaminated soils.

Keywords: Thallium, uptake, translocation, subcellular distribution, green cabbage

Introduction

Thallium (Tl), a non-essential trace metal that is detrimental to the food chain (Zitko 1975; Repetto, del Peso, and Repetto 1998), is classified as one of the 13 priority metal pollutants (Keith and Telliard 1979), and its pollution and associated health risks have aroused high concerns around the world (Madejon *et al.* 2007; Xiao *et al.* 2007; Vanek *et al.* 2010). Previous studies have showed that Tl in soil can be taken up easily by food crops, particularly by brassicaceous crops (Tremel *et al.* 1997; LaCoste Robinson, and Brooks 2001; Al-Najar *et al.* 2005). Our research group have also confirmed that, as a species of brassicaceous crops, green cabbage (*Brassica oleracea* L. *var. capitata* L.) can highly accumulate Tl from soils from a rural area of southwestern China, where Tl levels in soils are enhanced due to both mining and natural mineralization of Tl-rich sulfide minerals (Xiao *et al.* 2004; Jia *et al.* 2013). The concentration of Tl in leaves of the locally planted green cabbage can be up to 818 mg/kg (Jia *et al.* 2013). The elevated Tl contents in the local green cabbage are far above its occurrence (0.03–0.3 mg kg^{-1}) in food crops (Kabata-Pendias and Pendias 1992). Consequently, the average daily intake of Tl by the local villagers through consumption of locally planted crops is up to 1.9 mg per person (Xiao *et al.* 2004), which is a rate 1000 times higher than the world average daily intake (2 μg per day, Sabbioni, Ceotz, and Bingnoli 1984). As a result, the intake of high Tl through the locally planted green cabbages is a particular health problem to the local residents and has caused chronic Tl poisoning to the local residents (Xiao *et al.* 2007, 2012). On the other side, specifically, green cabbage (*Brassica oleracea* L. *var. capitata* L.) can be used to remove Tl from Tl-contaminated soils through phytoextraction.

Phytoextraction using plants to remove toxic trace metals from soils, sediments and water have been identified as a good alternative approach due to its environmental friendliness and relative ease of implementation. However, before using brassicaceous species to remediate Tl-contaminated soil, three scientific questions about green cabbage uptaking and accumulating Tl need to be well addressed: 1) what are the Tl accumulation characteristics of various green cabbage cultivars available in local market? 2) What are the Tl distribution characteristics at tissue level in green cabbage? 3) Which green cabbage cultivar has the highest tolerance and accumulation of Tl ? Although subcellular compartmentalization have been proposed as a major tolerance and accumulating mechanism

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for toxic elements in plants (Kwan and Smith 1991; Liu *et al.* 2009; Feng *et al.* 2015), the tolerance and accumulating mechanism of Tl in green cabbage is still unknown. Therefore, this study aimed to answer these questions by investigating the extent of Tl uptake, the translocation ability of Tl in tissues, and the subcellular distribution of Tl in the leaves of green cabbage cultivars. Further, the answers to these questions will be helpful for better understanding of the biochemical accumulation of Tl by various cultivars of green cabbage and the tolerance and detoxification of Tl in green cabbage, and will be beneficial for improving the agricultural management for cultivation of crops in Tl-polluted areas as well. Specifically, this study will contribute to accurately select green cabbage cultivars to phytoremediate Tl-contaminated soils.

Material and Methods

Soil Sampling and Preparation

The soil used in the pot trials was collected from the top layer (0–20 cm) of a sloping forest land without Tl pollution in the suburbs of Guiyang City (106°42′E, 26°34′N), Guizhou province, China. The outcropped rocks at the sampling sites are mainly composed of limestone and dolomite, and the collected soil is pedagogically classified as carbonate soil. The soil was passed through a 2-mm stainless-steel sieve to remove stones, plant roots, and other large particles, and then air-dried and homogenized.

The soil pH was measured after suspending soil in de-ionized water in a ratio of 1:2 (v/v) using a sensION156 pH-meter (Hach, USA). The content of total organic carbon (TOC) and total organic nitrogen (TON) was measured using an elemental analyzer PE2400–II (Perkin Elmer, USA). The cation exchange capacity (CEC) was computed after saturation of the soil samples with 0.005 M EDTA, 1 M ammonium acetate mixture, and a standard titration solution of ammonia acid. The mineral compositions were determined by X-ray diffraction (XRD) (D/Max-2200, Japan). Approximately 50 mg of the sieved soil sample (<74 μm) was digested using a heated acid mixture (15 mL of 15 M HNO_3 and 5 mL of 10 M HF) to determine Tl and other major elements.

Pot Trials

The collected soil was air-dried and passed through a sieve (<5 mm mesh), and divided into three groups of 50 kg each for pot experiments. Prior to soil sieving, we used a wooden roller to slightly break up the possible soil blocks to minimize the impact of soil properties to the pot trial. One group was left as a control (initial Tl at 0.56 mg kg^{-1}, Table 1) and the other two groups were then spiked with $TlNO_3$ solution (Merck, Germany) to achieve two Tl-contamination treatments with Tl concentrations approximately at 4.1 and 8.1 mg kg^{-1} (DW, based on ICP-MS analysis), respectively. The two Tl-spiked treatments were selected in order to appropriately meet to the bioavailable contents of Tl (less than 10 mg kg^{-1}) extracted with the weak acid (CH_3COONH_4) in the Tl-polluted soils of in southwestern China (Xiao *et al.* 2003). The soil sample of 2 kg from each group was transferred into 2.5 L plastic pot, and a total of 45 pots were applied. All the trial pots were then placed in dark corner of the greenhouse for two weeks, and around 50 mL Milli-Q water was periodically added to the soil to achieve the soil moisture content at around 25% and the geochemical equilibrium of Tl in pot soils.

Table 1. Physico-chemical properties of the soil used in the pot-culture trials.

Parameters	Results (n = 4) [A]
pH (1:2 v/v water)	7.31
Cation exchange capacity (CEC)	25.6 cmol kg^{-1}
Total organic carbon (TOC)	5.1%
Total organic nitrogen (TON)	0.54%
Ca	2.0%
Na	0.86%
K	1.2%
Mg	0.9%
Fe	3.4%
Mn	861 mg kg^{-1}
Tl	0.56 mg kg^{-1}
Mineralogical compositions	Quartz (93%)Feldspar (2.4%)Calcite (1.8%)Montmorillonite (1.8%)Goethite (1%)

[A] n: Number of soil samples

Five cultivars of green cabbage (Huifeng-1, Jingfeng-1, Sijiwang, Xinxiawang, and Zhonggan-19) that are commonly grown in China were selected for the pot trials. All seeds were separately germinated in 1-L pots containing the sieved soils from the control group without elevated Tl in a phytotron (RXZ–300C, Ningbo, China) at a temperature of 20–26°C and a relative humidity of 80% for around three weeks. The seedlings were fertilized with full-strength Hoagland's solution. The applied Hoagland solution contained 4 mM $Ca(NO_3)_2{\cdot}4H_2O$, 6 mM KNO_3, 1 mM $MgSO_4$, 2 mM $(NH_4)H_2PO_4$, and micro nutrients (46 μM H_3BO_3, 9 μM $MnSO_4$, 0.8 μM $ZnSO_4{\cdot}7H_2O$, 0.1 μM $Na_2MoO_4{\cdot}2H_2O$, 0.3 μM $CuSO_4{\cdot}5H_2O$, 50 μM EDTA-Na, and 50 μM $FeSO_4{\cdot}7H_2O$, and the pH was 6.4. All chemicals used in the experiments were of analytical grade. Three weeks after germination, one well-germinated seedling with four or five young leaves was transferred into each trial pot. All the three groups of treatments were replicated three times for each cultivar, and a total of 45 trials were applied. The plants were then grown in the greenhouse, with the temperature between 16 and 25°C (day 22–25°C, night 16–19°C) and relative humidity of 70–80%. After three months, three large leaves were collected from each plant by using a plastic knife prior to the whole plant cropping. Each cropped whole plant was partitioned into roots, stems, and leaves, which were then cleaned using de-ionized water to exclude any Tl contaminations by dusts or soil particles on the plants, and then air-dried in

labeled paper bags. At the time of harvesting, the potted plants were also examined to detect any visually evident sign of damage from Tl (e.g., chlorosis and leaf necrosis) and any apparent difference in size for each cultivar.

Chemical Analyses and Quality Control

All the collected samples of fresh leaves of green cabbage were washed several times using de-ionized water, and the water remaining on the leaves was removed by using tissue-papers. About 500 mg of fresh leaves collected from the center, tip and also the main leaf veins per plant were cut into small pieces and homogenized with a mortar and pestle to produce a single composite sample. The homogenizing solution was composed of 0.25 mM sucrose, 50 mM Tris-maleate buffer (pH = 7.8, the pH was adjusted by adding glacial acetic acid), 1 mM $MgCl_2$, and 10 mM cysteine (Weigel and Jäger 1980). All homogenizations were performed at 4°C in cooler. Each homogenized sample was frozen at –18°C for Tl concentration analysis in subcellular compartments within one day.

Separations of subcellular fractions were carried out according to the method reported by Weigel and Jäger (1980), with some modifications. The homogenate (around 15–20 mL) was transferred to a 50 mL centrifuge tube and centrifuged at 300 *g* for 15 min at room temperature using a high speed refrigerated centrifuge (Thermo Scientific Heraeus Megafuge 1.0 R). The resulting sediment (pellet) was designated as the cell wall fraction, consisting mainly of cell wall and cell wall debris. The supernatant was further centrifuged at 20,000 *g* for 45 min. The resultant deposition (pellet) was designated as the cell organelle fraction, and the resultant supernatant solution was referred as the cytosol and vacuole fraction. Each centrifuged subcellular fraction from each sample was transferred into a 50 mL glass beaker, respectively, and then digested with a 10-mL mixture of strong acids (8 mL of 15 M HNO_3 and 2 mL of 12 M $HClO_4$) for analysis of Tl and other major elements.

The remaining samples of fresh leaves collected from each plant were weighted, and then cut into small pieces (1–2 cm long) and oven-dried at 60°C until no further loss of weight. The dried samples, as well as the air-dried materials of roots, stems, and leaves of whole plant, were crushed and ground to fragments capable of passing through a 60-mesh screen using a crushing machine (FZ102, Taisite, China). Appropriately 100 mg of powder of each sample was digested in 10-mL mixture of strong acids (8 mL of 15 M HNO_3 and 2 mL of 12 M $HClO_4$) on a hotplate for analysis of Tl and other major elements.

The Tl concentrations of plant and soil digests were determined using inductively coupled plasma mass spectrometry (ICP−MS) (Perkin Elmer, ELAN DRC-e, USA), and the contents of major elements (K, Na, Mg, Ca, Fe, and Mn) were determined by inductively coupled plasma-optical emission spectrometry (ICP−OES, Varian, Vista MPX, USA) (Qi, Hu, and Grégoire 2000). The detection limit for Tl was 0.01 mg kg^{-1}, which is largely lower than the Tl contents in this study. The analytical precision, determined based on the standard quality control procedures using plant certified reference material of GBW07605 (tea powder, China National Research Center for Certified Reference Materials), internal standard (Rh at 500 μg/L), and duplicates, was better than ± 10%. The Tl content in the certified reference material GBW07605 was determined at 0.025 ± 0.001 mg kg^{-1} (n = 6), which is comparable with the certified value of 0.024 mg kg^{-1}, and a recovery of 104% was obtained. The determined data for plants were reported as dry weight (DW).

Statistical Analysis

The software package Analyse-it® (version 2.21, Analyse-it Software Ltd., Leeds, United Kingdom) was used for descriptive statistics, statistical tests and correlation analysis.

Results and Discussion

Soil Properties

The physico-chemical characteristics of the collected forest soil used in the pot-trials were summarized in Table 1. The forest soil had a pH value of 7.31 and a high Ca content of 2%, corresponding to the outcropped bedrock of carbonate at the sampling sites. The high TOC value of 5.1% resulted in a high CEC value of 25.6 cmol kg^{-1}. A high Fe content of 3.4% was observed in the soils. The Tl content in the forest soil was 0.56 mg kg^{-1}, similar to the Tl concentrations in the natural soils of the world (<1 mg kg^{-1}, Fergusson 1990) and China (0.29–1.2 mg kg^{-1}, Qi, Chen and Cao 1992). The mineralogical compositions in the soils were composed of quartz, feldspar, calcite, montmorillonite and goethite, and quartz was the dominant mineral (Table 1), implying the result of *in-situ* carbonate weathering.

Biomass of Green Cabbage

The biomass of green cabbage grown in the soils spiked with 0, 4.1, or 8.1 mg kg^{-1} Tl was plotted in Fig. 1. The biomass (fresh weight) ranged from 192.7 to 342.8 gram per pot (mean = 234.3 g, 95% CI = 218.9−249.8). The biomass showed no significant differences among cultivars ($p = 0.498$) or for individual cultivar with various treatments ($p = 0.152$). The leaves of cultivars under the three treatments were observed to be healthy without any obvious toxic symptoms during the pot-trials. This observation indicated that the enhanced Tl in the treated pot soil have not obviously affected the cabbage growth. Perhaps at higher Tl doses, variety differences might be observed.

Translocation of Tl in Tissues of Green Cabbage

The degree of translocation of a metal from root tissues to aerial parts can be described by translocation factor (TF) that is the ratio of metal concentration in plant's aerial parts and metal concentration in plant's root. A metal with a larger value of TF implies for its higher translocation capability from roots to stems and/or leaves. The TF values of Tl in the pot trials were listed in Table 2. The results showed that all the

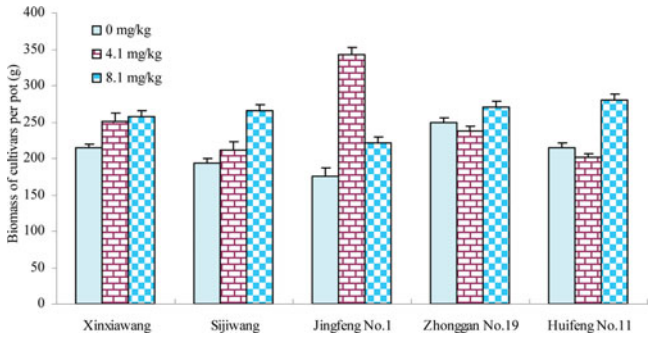

Fig. 1. Biomass of cultivars of green cabbage in pot-culture trials.

cultivars of green cabbage grown in the Tl-spiked pot soils had a high ability to translocate Tl from root to leaf, with the TF-leaf values ranging from 2.9 to 16.7. On the contrary, the stems of green cabbage had lower translocation ability, with the TF-stem values ranging from 0.27 to 0.95. No significant differences of TF-leaf values ($p = 0.691$) or TF-stem values ($p = 0.841$) were found among the different cultivars. This clearly revealed that green cabbage, particularly the leaf tissue, has high translocation ability for Tl. It also suggested that Tl is mobile enough to transfer from soil to the green cabbage, mainly into the leaf tissue. As observed in the pot-culture trials of this study, such high translocation ability also had no impact on the growth and biomass of green cabbage. The lower TF-stem values implied that the stem tissue, which is full of xylems and transmission catheter, acts as the transport channel for Tl from the soils to the leaves of green cabbage. The higher TF-leaf values suggested that the leaf is the major Tl storage-tissue in green cabbage.. This finding affirmed that green cabbage has high ability to accumulate Tl in leaf.

Table 2. Translocation factors (TF) of Tl in tissues of green cabbage.

Cultivars (n = 45) [A]	Spiked Tl in soil (mg/kg)	TF-stem [B]	TF-leaf [C]
Huifeng-1	0	/	/
	4.1	0.57	3.2
	8.1	0.95	12.6
Jingfeng-1	0	0.5	0.21
	4.1	0.51	4.5
	8.1	0.65	10.3
Sijiwang	0	0.13	0.14
	4.1	0.57	14.2
	8.1	0.34	2.9
Xinxiawang	0	0.24	0.1
	4.1	0.64	8.5
	8.1	0.46	16.7
Zhonggan-19	0	0.2	0.12
	4.1	0.38	8.6
	8.1	0.27	4.8

[A] n: Number of samples (Three samples for each treatment of individual cultivar)
[B] Tl in stem/Tl in soil.
[C] Tl in leaf/Tl in soil.

As a major storage site for Tl, the leaf of green cabbage contained elevated contents of Tl (Table 3). The total concentrations of Tl in the leaves of cultivars ranged from 101 to 192 mg kg^{-1} (mean = 136 mg kg^{-1}, median = 132 mg kg^{-1}, 95% CI = 114−157), and showed no significant differences among cultivars ($p = 0.455$). The determined Tl contents were far above the world average Tl level (0.02–0.3 mg kg^{-1}) for edible plants (Kabata-Pendias and Pendias 1992), and suggested that all the cultivars had high ability to uptake Tl from the Tl-polluted soils. Tl accumulation in the leaves of individual cultivar showed slight differences at the two Tl-spiked treatments ($p = 0.0291$), i.e., Tl averaged at 116 and 155 mg kg^{-1} at 4.1 and 8.1 mg kg^{-1} Tl-spiked treatments, respectively. The biological absorption coefficient (BAC, ratio of Tl concentration in plant to its concentration in the soils) values for all cultivars were slightly higher in the 4.1 mg kg^{-1} treatment than those in the 8.1 mg kg^{-1} treatment. The BAC values ranged from 25 to 37 (mean at 28) in the 4.1 mg kg^{-1} treatment, and from 16 to 24 (mean at 19) in the 8.1 mg kg^{-1} treatment. Analogous experiments with various vegetables have been carried out in New Zealand, and a similar result for BAC was reported at 34 for green cabbage grown in soil containing 3.7 mg kg^{-1} Tl (LaCoste *et al.* 2001). The above slight discrepancy in Tl accumulation between the two Tl-spiked treatments could be due to differed root-to-leaf translocation that is constrained by different Tl stresses.

Subcellular Distribution of Tl and Major Elements

Tl contents in subcellular fractions of leaf and the total Tl in leaf were summarized in Table 3. The recoveries were from 82% to 107%. The results showed significant differences ($p < 0.0001$) for the subcellular distribution of Tl in leaves of the green cabbage cultivars. The majority of Tl (85−92%) was observed in the cytosol and vacuole fraction, but only a minor part of Tl was associated with either the cell walls (6.6−12.6%) or the organelle fraction (1.3−2.3%). The results revealed a remarkable subcellular fractionation for Tl distribution in the leaves of green cabbages, i.e. cell cytosol and vacuoles >> cell walls > cell organelles. This specific subcellular fractionation of Tl clearly indicated that the cell cytosol and vacuoles in the leaf cells acted as a major storage site of Tl in green cabbage. This finding was consistent with other subcellular distribution studies of phytoaccumulation, in which the cell cytosol and vacuoles has been suggested to be the major site to store the uptaken metal in leaves (Bidwell *et al.* 2004; Liu *et al.* 2009).

A significant positive correlation ($R = 0.99$) between total Tl concentrations in leaves and Tl concentrations in the cell cytosol and vacuole fraction of green cabbage cultivars was also observed (Fig. 2). However, no significant differences in subcellular distribution of Tl were observed among the cultivars (p values were 0.508 for cell cytosol and vacuoles, 0.140 for cell walls, and 0.041 for cell organelles, respectively). In the controlled trial without Tl addition, Tl also generally showed the similar subcellular distribution pattern of cell cytosol and vacuoles > cell walls > cell organelles in leaves (Table 3).

The concentrations of the major elements in the whole leaf and individual subcellular fractions were plotted in Fig. 3.

Table 3. Summary of uptake and subcellular distribution of Tl in the leaves of selected green cabbage cultivars.

Cultivars (n = 45) [A]	Tl-spiked soil	Tl concentration (mg kg^{-1}, DW, mean±SD)				Recovery [B]	BAC [C]
		Cell wall fraction	Cell organelles fraction	Cytosol and vacuole fraction	Total		
Huifeng-1	0	n.d.[D]	n.d.	n.d.	n.d.		
	4.1	12.1 ± 2.1 (12.1%).[E]	1.9 ± 0.5 (1.9%)	85.5 ± 19.8 (86%)	110 ± 12.5	90%	27
	8.1	8.5 ± 3.7 (6.6%)	1.7 ± 0.8 (1.3%)	119.6 ± 29.8 (92.1%)	127 ± 56.4	102%	16
Jingfeng-1	0	0.2 ± 0.1	n.d.	0.4 ± 0.1	0.75 ± 0.1		
	4.1	11.1 ± 1.8 (11.5%)	1.6 ± 0.4 (1.7%)	83.8 ± 16.4 (86.8%)	105 ± 26.2	92%	26
	8.1	13.2 ± 2.0 (9.6%)	1.9 ± 0.3 (1.4%)	122.0 ± 11.4 (89%)	152 ± 35	91%	19
Sijiwang	0	0.2 ± 0.1	n.d.	0.4 ± 0.1	0.59 ± 2.1		
	4.1	17.4 ± 3.6 (11.8%)	2.7 ± 0.6 (1.8%)	127 ± 28.2 (86.4%)	152.0 ± 12	97%	37
	8.1	15.8 ± 4.1 (10%)	3.1 ± 0.1 (2%)	139 ± 15.7 (88%)	192 ± 33.8	82%	24
Xinxiawang	0	0.1 ± 0.1	n.d.	0.7 ± 0.1	0.78 ± 0.3		
	4.1	9.6 ± 3.6 (9.3%)	1.4 ± 0.5 (1.4%)	92.7 ± 6.9 (89.3%)	112 ± 47.8	93%	27
	8.1	12.8 ± 2.8 (8.9%)	2.1 ± 0.4 (1.4%)	128 ± 19.8 (89.7%)	168 ± 58.1	85%	21
Zhonggan-19	0	0.1 ± 0.1	n.d.	0.3 ± 0.1	n.d.		
	4.1	11.6 ± 7.6 (10.8%)	2.2 ± 1.6 (2%)	93.6 ± 16.4 (87.2%)	101 ± 48.7	107%	25
	8.1	15.2 ± 0.8 (12.6%)	2.8 ± 0.7 (2.3%)	102 ± 11.2 (85.1%)	137 ± 13.1	88%	17

[A] n: Number of samples (Three samples for each treatment of individual cultivar)
[B] (Tl in cell wall + cell organelles + cell cytosol and vacuole)/(Total Tl in leaves) × 100.
[C] Biological absorption coefficient: ratio of Tl concentration in plant to its concentration in the rhizosphric soils.
[D] Not detectable.
[E] The figures of percentage in the parentheses refer to the subcellular contribution of Tl in leaf cells.

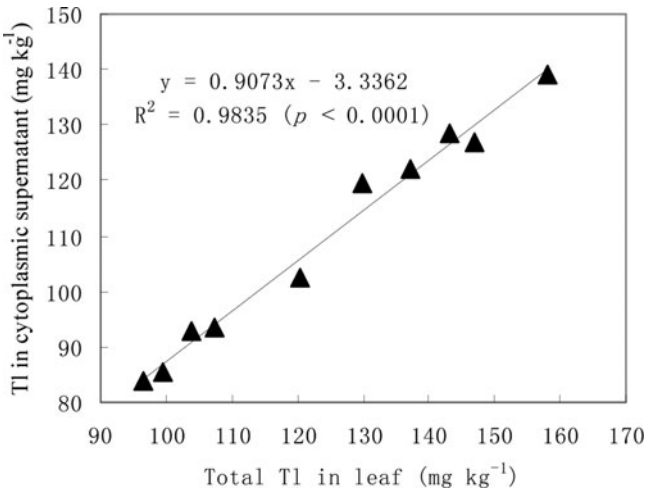

Fig. 2. Correlation between Tl contents in leaf and in the cytosol and vacuole fraction of green cabbage cultivars.

With the exception of the cell organelles, the relative magnitudes of K, Na, Mg, Ca, Fe, and Mn were similar in every subcellular fraction, although their actual values were different among the subcellular fractions. The average concentrations of each major element in the leaf cells of green cabbage also represented subcellular fractionation: the cell cytosol and vacuole faction > the cell wall fraction > the cell organelle fraction, which indicated that the cell cytosol and vacuole were also the major storage sites for the major elements.

Positive correlations between Ca and Tl were observed in the cell cytosol and vacuoles ($R = 0.38$), the cell walls ($R = 0.34$), and the whole leaf ($R = 0.33$) (Table 4). Similar positive correlations between Mg and Tl were also obtained in cell cytosol and vacuoles ($R = 0.37$) and the whole leaf ($R = 0.48$). These findings were consistent with the previous observations that a higher accumulation of Tl in crops growing in Tl-polluted soils in field generally corresponded to elevated concentrations of Ca and Mg (Xiao *et al.* 2004).

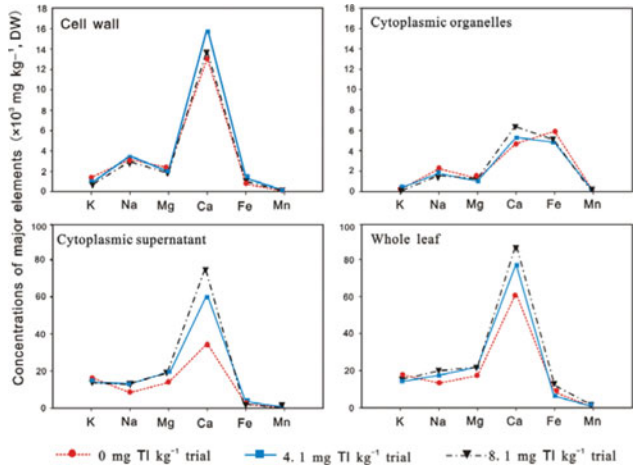

Fig. 3. Subcellular fractionation patterns of major elements in leaves of green cabbage from the pot-culture trials.

Table 4. Pearson's correlation coefficients matrix between Tl and major elements.

Tl in subcellular fractions	K	Na	Mg	Ca	Fe	Mn
Cell wall ($n = 45$) [A]	−0.28	0.26	−0.01	0.34*[B]	0.23	0.71*
Cell organelles ($n = 45$)	−0.09	−0.12	0.10	0.21	−0.16	0.35*
Cytosol and vacuole ($n = 45$)	−0.21	0.56*	0.37*	0.38*	0.07	0.77*
Whole leaf ($n = 45$)	0.02	0.51*	0.48*	0.33*	0.23	0.60*

[A] n: Number of samples.
[B] *: Significant at $p < 0.05$.

Moreover, a positive correlation between Tl and Ca in the main vein of the basal leaves of *Iberis intermedia* was also observed by means of optical imaging and X-ray fluorescence maps (Scheckel *et al.* 2004). These studies suggested that Ca may play an important role in accumulation/tolerance of Tl in green cabbage.. In addition, significantly positive correlations between Tl and Mn and Na, particularly in the cell cytosol and vacuoles ($R_{Mn} = 0.77$, and $R_{Na} = 0.56$), were observed for the first time (Table 4). In contrast, K was negatively correlated with Tl in the three subcellular fractions, and a near-zero correlation for the whole leaf, but none of these correlations were significant (Table 4). This could be attributable for the substitution of Tl for K in plant cells due to similar ionic radii of Tl^+ (1.59 Å) and K^+ (1.51 Å) (Siegel and Siegel 1976). This finding was comparable to the experiment result of Madejon *et al.* (2007), who found no significant correlation between K and Tl in various parts of two brassicaceous plants. However, the biological roles of the above elements in accumulation of Tl in leaf of green cabbage as well as in tolerance of green cabbage for Tl are still unclear and remain further investigation.

This study clearly demonstrated that the crop species of green cabbage has high ability to accumulate Tl, although Tl is non-essential to plants (Repetto *et al.* 1998). However, the highly accumulated Tl (101–192 mg kg^{-1}, Table 3) did not result in growth inhibition and toxicity symptoms of green cabbage from either the pot-culture trials of this study or the field (Xiao *et al.* 2004). The biomass also appeared to have no significant differences among various Tl-spiked treatments ($p = 0.152$) (Fig. 1). This suggested that green cabbages must have high resistance to Tl toxicity. The high tolerance for Tl of green cabbage was likely attributable for the subcellular fractionation of Tl in leaf. As discussed above, the majority (~ 88%) of leaf-Tl was stored in the cell cytosol and vacuole fraction, which represented as the storage site of Tl in leaf of green cabbage. Thus, the large storage capacity of cell cytosol and vacuole for Tl may be the main factor contributing to the tolerance or detoxification for Tl in leaf of green cabbage.

According to Hall (2002), the ability of cytosol and vacuoles in leaf cell to preferentially compartmentalize Tl must facilitate the green cabbage to avoid Tl toxicity damage to its vital organelles. The chelation of Tl in the cytosol by peptides

such as phytochelatins could be a potential cellular mechanism that may be involved in the detoxification of Tl and thus tolerance to Tl stress (Hall 2002). Moveover, vacuole is considered to be the final destination for practically all toxic substances exposed to plants (Clemens 2006; Peng and Gong 2014), and excess (non-)essential metals are sequestered in leaf cell vacuoles and translated into low toxic complexes (Clemens, Palmgren, and Krämer 2002). Similarly, this specific vacuolar compartmentalization is also suggested as an important cellular mechanism of detoxification for trace metals (Hall 2002). Further, similar with other heavy metals of Zn, Cd, Mo and Ni (Brune, Urbach, and Dietz 1995), transportation of Tl into the vacuole could be a potential way of reducing the levels of Tl in the cytosol and thus is potentially important for Tl tolerance in the leaf cells of green cabbage. This strategy attempts to avoid the build-up of excess metal levels in the cytosol, and thus delays or prevents the onset of toxicity symptoms (Hall 2002). Similar vacuolar compartmentalization was reported that 80% of Tl uptaken by *Lemna minor* L. (common duckweed) was stored in the cell vacuoles (Kwan and Smith 1991). Therefore, the subcellular compartmentalization in the cell cytosol or/and the cell vacuole play(s) a vital role in the tolerance and detoxification of Tl in leaf of green cabbages, although Tl contents in the leaf cell cytosol and the leaf cell vacuole were not separately determined in this study. In addition, quite lower Tl contents (1.3−2.3% of leaf-Tl, Table 3) associating with the cell organelle fraction were also significant for resulting in low phytotoxicity to the cell organelles. The quantitative roles of these components in Tl-binding as well as their properties in leaf of green cabbage remain further investigations.

Conclusions

The pot-culture trials of this study showed that all the five green cabbage cultivars represented high translocation ability for Tl in leaf (2.9–16.7) but low translocation ability in stem (0.27–0.95). All the selected cultivars highly accumulated Tl in leaves with high biological absorption coefficients (16–37). The subcellular distribution of Tl in leaves of green cabbage showed obvious compartmentalization, with concentration decreasing in the order of cytosol and vacuole >> cell wall > cell organelles. Similar subcellular fractionations were also found for major elements. The majority (~ 88%) of leaf-Tl was located in the cytosol and vacuole fraction, with positive correlations with Ca, Mg, Mn and Na. This specific subcellular fractionation appeared to facilitate detoxification of Tl by keeping it away from sensitive cellular organelles in leaf cells. All the five green cabbage cultivars are alterative materials to phytoremediate Tl-contaminated soils.

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