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The use of experimental data and the application of a kinetic model to determine the subcellular distribution of Zn/Cd/Ni/Cu over time in Indian mustard

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The precise subcellular distribution of metallic elements in plants may have a significant impact on metal detoxification and bioaccumulation processes. In this study, we report the subcellular distribution of metallic elements in Indian mustard (*Brassica juncea*) and the application of a kinetic model to confirm the experimental data by aqua-culture experiment. The metals in Indian mustard were determined on the 3rd, 5th, 9th, and 14th day. The results showed that concentrations of Zn, Cd, and Ni in root samples increased with exposure time, reaching maximum values of 13 161, 9602, and 864 mg kg^{-1} (DW), respectively, at the 9th day, while concentrations of Cu in root samples reached a maximum value of 17 566 mg kg^{-1} (DW) on the 14th day. A decrease in the concentration of Cu/Zn/Cd/Ni from roots to stem/leaf was observed, however, the difference in metal concentration between the roots and stem/leaf was much greater for Cu than for the other three metals. The majority of these metals were present in the cell walls, organelles, and the soluble fraction, with only minor amounts present in the cell membranes. The Zn, Cd, Ni and Cu existed mainly in the cell walls of the root on the 14th day, reaching maximum values of 424, 483, 23 and 839 mg kg^{-1} (fresh samples), respectively. Nearly all (90%) of the Cu existed in the cell water-soluble fraction of stems and leaves on the 3rd day, however, 44%–52% of Cu was present in the cell water-soluble fraction on the 14th day. There were also 25%–35% of Zn, 53%–60% of Cd and 70%–73% of Ni in the cell water-soluble fraction of stems and leaves. The kinetic model adequately described the experimental data for accumulation by roots, stems, and leaves. The majority of the data for the subcellular fractions of roots and stems fit the equations well. The accumulation rate constant k and the maximum accumulation capacity y_{max} of plant and subcellular fractions were calculated. Our results provide insight into the accumulation and subsequent subcellular distribution of metals in plants in hydroponics culture and will be valuable for further studies of phytoremediation.

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Introduction

The pollution of soil and aquatic ecosystems with metallic elements is a serious environmental problem because these elements are persistent in the environment and are highly toxic to most organisms.[1–3] To avoid metal toxicity, plants have developed intra- and extracellular mechanisms for metal detoxification, such as binding and precipitation in the cell wall and/or compartmentalisation in vacuoles.[4] There is some evidence that the subcellular distribution of metallic elements may be associated with metal tolerance and detoxification in plants.[5] Ramos *et al.* observed that the Cd in contaminated lettuce plants was largely present in the cell wall fraction,[6] and a similar subcellular distribution pattern has been reported in ramie.[7] Understanding the processes of metal accumulation and subcellular distribution in plants is essential for the assessment of crop contamination, human exposure, and subsequent phytoremediation.

Phytoremediation processes have been developed based on studies of metal tolerance in plants and typically employ high-biomass plant species exhibiting rapid growth and hyperaccumulation properties, such as oat (*Avena sativa*), barley (*Hordeum vulgare*), and Indian mustard (*Brassica juncea*).[8–11] Indian mustard has been identified as a high biomass-producing plant with the capacity to accumulate metallic cations at high concentrations in its cells. The phytotoxicity of a particular metal is partially determined by its biological activity in the plant, which is associated with its subcellular distribution.[12–14] Relatively little is known about the subcellulars and activities of metallic cations such as Zn, Cd, Ni, and Cu in plants. Therefore, it is important to determine the subcellular distribution of these metallic elements in plants

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such as Indian mustard because this factor influences the characteristics of metal migration and accumulation and thus the potential effectiveness of phytoremediation processes.

Some plants can tolerate soils that are contaminated with metallic elements and even accumulate these elements. This accumulation ability may depend on how the plant distributes the toxins in its tissues and the concentration of metals that are taken up. Furthermore, the phytoremediation capacity of a plant has a significant correlation with its maximum accumulation capacity, which must be known in order to develop a mechanistic model for the effects of changes in environmental conditions on metal uptake.[15–17] In recent studies by Guala *et al.*, a kinetic model was constructed to predict potential metal phytoextraction ability. The maximum accumulations of metallic elements in the harvestable parts of plants could be estimated using the proposed kinetic model.[18,19] Metal accumulation and bioaccumulation kinetics show substantial variability depending on the species, metallic element, and ecosystem investigated.[20] Due to this complex behaviour, few mechanistic accumulation and bioaccumulation models for metallic elements are available, and plant accumulation and subcellular distribution models are particularly lacking. Once these models are developed, factors such as maximum accumulation capacity and accumulation rate constant can be predicted, which in turn can help guide phytoremediation programs.

In the present study, we investigated the accumulation and subcellular distribution of Zn, Cd, Ni, and Cu in Indian mustard and evaluated the plant's ability to sequester these metals from solution and preferentially store them in the roots, stems, and leaves at the cellular and subcellular level. We also predicted two kinetic parameters, the accumulation rate constant k and the maximum accumulation capacity y_{max}, for plant and subcellular fractions. Our results add to what is known about metal transfer and transformation in Indian mustard and will be valuable for use in risk assessments of metal contamination at polluted sites.

Experimental

Plant material and growth conditions

The Indian mustard (*Brassica juncea* L. var. *megarrhiza*) seeds used were provided by the College of Life Science of Nanjing Agricultural University, China. The seeds were germinated, and grown in 20 cm deep water containers in a greenhouse equipped with supplementary lighting (14 h photoperiod) and with day and night temperatures of 25–35 and 15–25 °C, respectively. After approximately 5 days, healthy plants of the same size (with 3–4 leaves) were chosen, cleaned, and cultured in 5 L plastic pots (20 cm tall with 14 plants per pot) containing 1/4 strength Hoagland nutrient solution containing (mmol l^{-1}) 2.00 $Ca(NO_3)_2{\cdot}4H_2O$, 0.10 KH_2PO_4, 0.50 $MgSO_4{\cdot}7H_2O$, 0.10 KCl, 0.70 K_2SO_4; (μmol L^{-1}) 10.00 H_3BO_3, 0.50 $MnSO_4{\cdot}H_2O$, 1.00 $ZnSO_4{\cdot}7H_2O$, 0.20 $CuSO_4{\cdot}5H_2O$, 0.01 $(NH_4)_6Mo_7O_{24}$, and 10 Fe-HBED.[21,22] The nutritive solution was continuously aerated and renewed every 4 days, and the pH was adjusted to 6.1–6.2 with 0.1 mol l^{-1} HCl or 1 mol l^{-1} NaOH every 2 days.[23] The total volume of the solution was kept constant by adding deionised water to compensate for water lost through plant transpiration, sampling, and evaporation. The medium was aerated to achieve constant stirring of the solution.

Plant accumulation experiment

After acclimatisation for 2 weeks, the plants were approximately 14 cm tall with relatively mature roots. Plants were harvested on the 3rd, 5th, 9th, and 14th day after receiving treatments of 100 μmol l^{-1} $ZnCl_2$, $CdCl_2$, $NiCl_2$, or $CuCl_2$ in Hoagland nutrient solution separately. Fourteen seedlings were cultured in separate plastic pots with three replicates for each time point and each metal treatment. During the experimental period, the seedlings were grown in a greenhouse with day and night temperatures of 25–35 and 15–25 °C, respectively.

After the indicated period of exposure to the metallic elements, all plants were completely harvested from each plastic pot. The roots were immersed in 20 mmol l^{-1} Na-EDTA to release the adsorbed metallic elements. Then the whole plant was rinsed with deionised water and separated into roots, stems, and leaves, which were then frozen in liquid N_2 until use.[24]

Subcellular fractions

A 2000 mg sample of frozen material was homogenised in cold extract buffer containing 50 mM HEPES, 500 mM sucrose, 1.0 mM dithiothreitol (DTT), 5.0 mM ascorbic acid, and 1.0% (w/v) poly(vinylpyrrolidone) (PVP, K90, average molecular weight: 1 300 000), and adjusted to pH 7.5 with NaOH. Cells were separated into cell wall, water-soluble, organelle, and cell membrane fractions using the differential centrifugation technique described by Lozano-Rodriguez *et al.*[25] with some modifications. The homogenate was sieved through a nylon cloth (100 μm mesh size) and washed with extraction buffer. This residue, together with the pellet produced by centrifugation of the filtrate at 100 g for 5 min, constituted the cell wall-bound metals (Fraction A). The supernatant contained the remaining metals, which were further separated into three fractions. First, the supernatant was centrifuged at 10 000*g* for 30 min, producing a pellet containing the organelles (Fraction B). The supernatant from that centrifugation was then centrifuged at 100 000*g* for 30 min (Beckman, Optima L-100XP), producing a pellet containing the cell membranes (Fraction C). The remaining supernatant contained the soluble fraction (Fraction D). All steps were performed at 4 °C.

Analysis of metallic elements

Before the metal analysis, 5 μl concentrated HNO_3 was added to each 1 ml sample solution. The plant materials were dried at 105 °C for 24 h and weighed to determine the dry weight (DW). All plant materials and the centrifugation fractions A, B, C and D were wet digested in concentrated HNO_3 : $HClO_4$ (7 : 3, v/v). The metal concentrations of the plant materials and plant subcellular fractions were determined using ICP-OES (ICP-OES, Optima 2000DV, Perkin-Elmer Co., USA).

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Data analysis and kinetic model application

Data analysis was carried out using the Origin software package. The metal accumulation and distribution in the roots, stems, leaves, and subcellular fractions over time were estimated by fitting a two-parameter curve using nonlinear regression. The accumulation process can be defined in the following way using a general form of the equation for metal accumulation:

$$\frac{dy}{dt} = k(y_{max}\text{-}y) \quad (1)$$

where y represents the concentration in the plants, y_{max} is the maximum accumulation capacity (mg kg^{-1}), k is the accumulation rate constant (d^{-1}), and t is time (d). This indicates that the rate of metal accumulation is dependent on the accumulation rate constant and accumulation capacity. After solving eqn (1), the three-parameter curve can be expressed as:

$$y = y_{max} - A_1\exp(-kt) \quad (2)$$

The equation shows that the initial accumulation of metallic elements is zero at $t = 0$. Then, eqn (2) can be solved:

$$y = y_{max} - y_{max}\exp(-kt) \quad (3)$$

Statistical analysis

All data were evaluated using Excel 2003 and SPSS. Every data point shown in the figures represents an average value. The standard deviations (SD) obtained from three parallel samples are shown in the figures as error bars. The data were analysed using analysis of variance (ANOVA) in SPSS (Version 13.0) with a confidence limit of 95%.

Results

Accumulation of metallic elements over time by Indian mustard

On the 14th day after metal exposure, the Indian mustard plants had a healthy appearance, and no mortality occurred. No significant difference in plant biomass was recorded between the four different metal treatments. The accumulation of metallic elements by Indian mustard during the exposure phase was nonlinear for all treatments (Fig. 1). The concentrations of Zn, Cd, and Ni in root samples increased with exposure time, reaching maximum values of 13 161 $\pm$ 2946, 9602 $\pm$ 730, and 864 $\pm$ 75 mg kg^{-1} (DW), respectively, on the 9th day, while concentrations of Cu in root samples reached a maximum value of 17 566 $\pm$ 103 mg kg^{-1} (DW) on the 14th day. The concentrations of Zn and Cd in the roots decreased from the 9th to 14th day, whereas the concentrations of these metals in the leaf samples continued to increase from the 3rd to 14th day.

The trends shown in Fig. 1 indicate declining concentrations of Cu/Zn/Cd/Ni from the roots to the stem/leaf; however, the difference in metal concentration between the roots and stem/leaf was much greater for Cu than for the other three metals. The Cu concentration in the roots reached an apparent maximum accumulation value at the 14th day, which was higher than the levels of the other three metals.

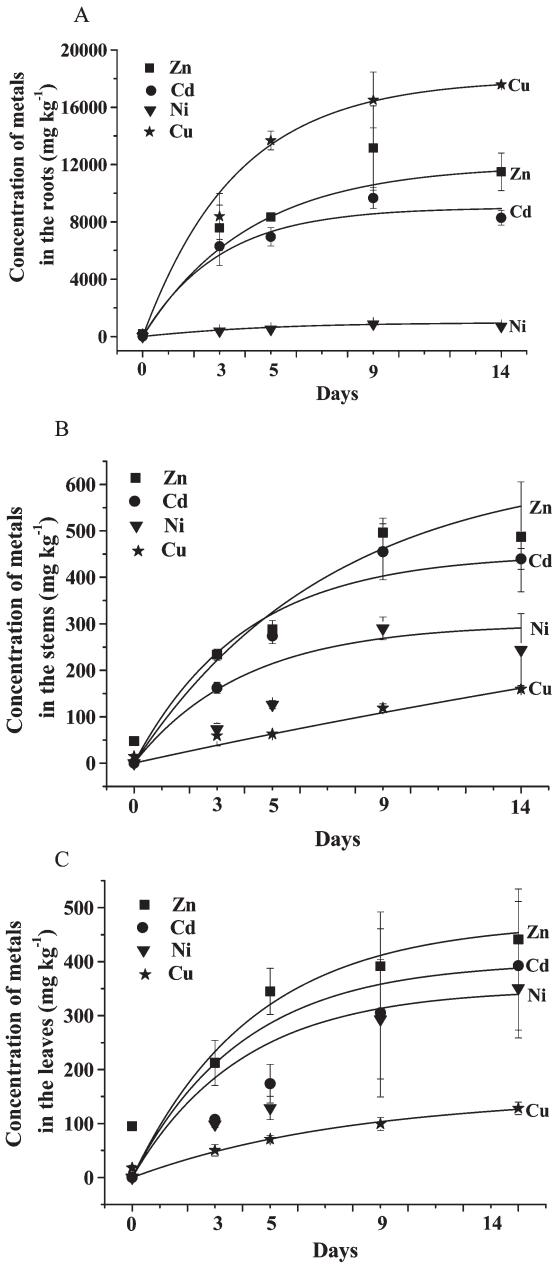

Fig. 1 Concentrations of metallic elements in roots (A), stems (B), and leaves (C) of Indian mustard as a function of time. The concentrations in the solution applied were all 100 μmol l^{-1}. The solid lines show the kinetic model prediction based on eqn (1).

For each metallic cation, the stem and root, but not leaf, accumulation data fitted eqn (3) well and produced significant R^2 values. The accumulation rate constant k and maximum accumulation capacity y_{max} was calculated for the experiment. A significant correlation between the values of y_{max} and the observed metal accumulation by plants was found.

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Table 1 Estimates of kinetic parameters for metal accumulation in different fractions

		Roots			Stems			Leaves		
Metal	Fraction	R^{2a}	k^b	$y_{max}{}^c$	R^2	k	y_{max}	R^2	k	y_{max}
Zn	Total[d]	0.91	0.28	12 582	0.90	0.13	598	0.70	0.06	440
	Soluble	0.76	0.31	48	0.77	0.92	8	—	—	—
	Organelles	0.88	0.40	124	0.67	0.04	15	0.94	0.05	15
	Wall	0.98	0.36	406	—	—	—	0.91	0.10	23
	Membranes	—	—	—	—	—	—	—	—	—
Cd	Total	0.93	0.38	8925	0.81	0.12	575	0.85	0.78	381
	Soluble	0.91	0.39	40	—	—	—	—	—	—
	Organelles	0.83	0.48	151	0.99	0.11	3	—	—	—
	Wall	0.99	0.32	477	—	—	—	—	—	—
	Membranes	—	—	—	—	—	—	—	—	—
Ni	Total	0.86	0.23	827	0.67	0.14	335	0.77	0.43	402
	Soluble	0.99	0.15	32	—	—	—	—	—	—
	Organelles	0.85	0.12	4	0.56	33.33	0.8	0.65	23.26	1
	Wall	1.00	0.28	23	0.66	0.09	8	—	—	—
	Membranes	—	—	—	—	—	—	0.60	20.00	0.7
Cu	Total	0.99	0.23	18 588	0.87	0.04	324	0.76	0.39	110
	Soluble	0.87	0.22	350	—	—	—	—	—	—
	Organelles	0.78	0.49	227	—	—	—	—	—	—
	Wall	0.88	0.42	734	—	—	—	0.87	0.09	6
	Membranes	—	—	—	—	—	—	—	—	—

[a] Coefficients. [b] Accumulation rate constant (d^{-1}). [c] Maximum accumulation capacity (mg kg^{-1}). [d] Total concentration of metallic elements in roots, stems or leaves (mg kg^{-1}). — = no significant correlation.

Subcellular distribution of metallic cations over time in Indian mustard

The distribution of the metallic cations in the roots, stems, and leaves in four subcellular fractions (the water-soluble, cell wall, organelle, and cell membrane fractions) was investigated. The measured concentrations of metallic cations in each fraction are shown in Tables 2–4.

Metallic elements accumulated in the cell walls, soluble fractions, organelles, and membranes after 14 days of exposure. However, the concentrations in the subcellular fractions of each tissue differed significantly during the exposure period. The Zn, Cd, Ni and Cu existed mainly in the cell walls of root on the 14th day, reaching maximum values of 424 $\pm$ 14, 483 $\pm$ 22, 23 $\pm$ 1 and 839 $\pm$ 550 mg kg^{-1} (fresh samples), respectively. The concentrations of Zn and Cd in the soluble fraction of the roots were highest after 9 days of exposure, whereas those of the stems and leaves were highest after a 14-day exposure period. The concentrations of Ni in the soluble fraction of the roots, stems and leaves were highest on the 14th day. The concentrations of all four metals in the cell wall and organelle fractions of the stems and leaves were highest on the 14th day. Small amounts of Zn and Ni accumulated in the cell membrane fractions of the roots, stems, and leaves, but no significant accumulation of Cd or Cu was found in any cell membrane fraction.

With the exception of the cell membrane fraction, the patterns of accumulation in the subcellular fractions of the root samples fit eqn (3) well and produced significant R^2 values. In contrast, the pattern of Cu accumulation in all subcellular fractions of the stem samples did not fit eqn (3) well. Similarly, the patterns for all metals in the membrane fractions of stem samples and in most of the subcellular fractions of leaf samples also did not fit eqn (3) well. The accumulation rate constant k and maximum accumulation capacity y_{max} calculated for the subcellular fractions are shown in Table 1.

Proportions of metallic elements in each subcellular fraction

The proportions of metallic elements in each subcellular fraction are plotted against time in Fig. 3. The majority of metallic elements were associated with the cell wall, organelle, and water-soluble factions with only minor amounts present in cell membranes. For the root samples, the Zn, Cd, Ni and Cu existed mainly in the cell walls on the 14th day. In the stem samples, the majority of the Zn was present in the water-soluble fraction and the cell walls. Nearly 40–50% of the Zn was present in the cell water-soluble fraction after the 5th day, but this number later decreased to approximately 24%, whereas the majority of the Cd, Ni, and Cu remained in the cell water-soluble fraction throughout the experimental period. Nearly all (93.2%) of the Cu existed in the cell water-soluble fraction on the 3rd day, and 59.6% of Cd and 73.1% of Ni was present in the cell water-soluble fraction on the 14th day. There were also 25% of Zn, 44% of Cu in the cell water-soluble fraction of stems on the 14th day. For the leaf samples, the higher concentration of Ni was present in the cell water-soluble fraction after 9 days of exposure. Zn and Cd were present in the cell water-soluble fraction and in the cell walls, whereas Cu was present mainly in the water-soluble fraction on the 3rd day (96.7%) and then decreased to 52.2% on the 14th day. There were also 35% of Zn, 53% of Cd, and 70% of Ni in the cell water-soluble fraction of leaves on the 14th day.

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Table 2 Metal concentrations in each cell fraction per unit (kg) roots over time following exposure to 100 μmol l^{-1} metal solution

		Amount of metallic element in each cell fraction per unit (kg) roots (mg kg^{-1} root)				
Metal	Days	Soluble fraction	Cell wall	Organelle	Membrane	Recovery[b] (%)
Zn	3	31.99 ± 12.14[a]	279.45 ± 1.51	72.14 ± 1.24	0.59 ± 0.80	92.4
	5	30.92 ± 1.94	337.94 ± 42.66	127.72 ± 12.84	3.05 ± 0.76	83.7
	9	55.84 ± 4.73	366.68 ± 24.97	116.27 ± 19.30	2.93 ± 0.19	85.6
	14	42.12 ± 7.72	424.14 ± 14.32	119.13 ± 16.84	0.86 ± 0.51	92.1
Cd	3	24.98 ± 4.25	309.11 ± 4.41	95.95 ± 1.70	2.76 ± 0.63	92.8
	5	35.50 ± 10.88	371.56 ± 3.43	169.33 ± 16.14	3.44 ± 0.63	93.6
	9	43.38 ± 9.47	440.36 ± 29.86	147.35 ± 19.94	3.33 ± 1.44	94.1
	14	35.13 ± 6.58	483.12 ± 22.21	139.50 ± 18.03	0.84 ± 0.24	95.7
Ni	3	10.89 ± 5.55	13.55 ± 1.25	0.97 ± 0.02	1.06 ± 0.04	94.2
	5	18.17 ± 3.80	17.44 ± 2.57	2.60 ± 0.39	0.60 ± 0.04	91.6
	9	22.77 ± 12.03	21.21 ± 0.62	2.57 ± 0.16	0.64 ± 0.05	87.5
	14	28.30 ± 7.35	23.07 ± 1.37	3.75 ± 0.07	0.66 ± 0.09	95.4
Cu	3	111.42 ± 1.12	453.54 ± 3.38	159.82 ± 0.71	19.75 ± 0.23	93.7
	5	268.52 ± 61.72	716.39 ± 91.54	242.46 ± 29.91	13.49 ± 1.49	88.4
	9	321.18 ± 50.32	795.51 ± 55.49	175.69 ± 3.45	13.04 ± 2.48	93.4
	14	311.69 ± 107.35	838.55 ± 549.66	254.45 ± 34.16	4.57 ± 2.10	96.6

[a] Mean ± standard deviation (n = 3). [b] Percentage recovery (%) = (cell wall + organelle + soluble fraction + membrane) × 100%/total.

Table 3 Metal concentrations in each cell fraction per unit (kg) stems over time following exposure to 100 μmol l^{-1} metal solution

		Amount of metallic element in each cell fraction per unit (kg) stems (mg kg^{-1} stems)				
Metal	Days	Soluble fraction	Cell wall	Organelle	Membrane	Recovery (%)
Zn	3	7.79 ± 0.24	15.20 ± 4.29	3.49 ± 0.03	1.55 ± 0.72	92.1
	5	8.78 ± 0.75	5.90 ± 0.52	3.17 ± 0.05	0.07 ± 0.02	86.7
	9	6.38 ± 1.80	13.19 ± 0.28	3.45 ± 0.30	8.95 ± 0.73	90.9
	14	10.11 ± 1.06	22.45 ± 0.82	6.90 ± 1.83	0.24 ± 0.01	91.7
Cd	3	0.52 ± 0.07	1.86 ± 0.09	0.96 ± 0.02	0.00 ± 0.00	89.1
	5	4.09 ± 0.19	1.75 ± 0.17	1.26 ± 0.33	0.00 ± 0.00	88.4
	9	9.59 ± 0.12	5.57 ± 0.92	2.04 ± 0.80	0.00 ± 0.00	89.2
	14	31.40 ± 5.40	18.67 ± 0.33	2.47 ± 1.61	0.15 ± 0.10	91.6
Ni	3	4.52 ± 1.62	3.69 ± 0.56	1.06 ± 0.21	0.94 ± 0.13	89.1
	5	4.71 ± 0.03	3.18 ± 0.87	0.57 ± 0.04	0.46 ± 0.05	91.5
	9	12.31 ± 2.60	3.63 ± 0.09	0.66 ± 0.01	0.50 ± 0.07	87.2
	14	21.87 ± 2.54	6.67 ± 0.65	0.79 ± 0.12	0.58 ± 0.05	93.4
Cu	3	46.63 ± 7.95	3.08 ± 0.26	0.32 ± 0.05	0.00 ± 0.00	85.8
	5	9.38 ± 4.44	1.81 ± 0.47	0.19 ± 0.03	0.00 ± 0.00	92.6
	9	2.69 ± 0.82	2.66 ± 0.33	0.42 ± 0.04	0.00 ± 0.00	93.4
	14	7.20 ± 0.95	7.86 ± 3.29	1.23 ± 0.14	0.00 ± 0.00	92.8

Table 4 Metal concentrations in each cell fraction per unit (kg) leaves over time following exposure to 100 μmol l^{-1} metal solution

		Amount of metallic element in each cell fraction per unit (kg) leaves (mg kg^{-1} leaves)				
Metals	Days	Soluble fraction	Cell wall	Organelle	Membrane	Recovery (%)
Zn	3	10.56 ± 2.84	8.44 ± 1.34	3.31 ± 0.78	1.71 ± 1.17	94.7
	5	6.88 ± 0.46	7.95 ± 0.84	3.41 ± 0.92	0.71 ± 0.02	87.3
	9	11.57 ± 2.34	12.81 ± 2.78	5.49 ± 0.35	0.81 ± 0.12	91.6
	14	14.43 ± 2.26	17.59 ± 3.74	8.23 ± 2.06	0.44 ± 0.52	87.9
Cd	3	1.50 ± 0.16	3.17 ± 0.40	0.15 ± 0.04	0.00 ± 0.00	92.4
	5	3.93 ± 1.87	3.01 ± 0.31	1.93 ± 0.79	0.00 ± 0.00	89.6
	9	17.71 ± 0.93	9.12 ± 1.93	4.95 ± 0.28	0.00 ± 0.00	84.3
	14	27.22 ± 1.76	20.25 ± 2.61	2.91 ± 0.10	0.30 ± 0.12	89.1
Ni	3	4.69 ± 1.13	3.82 ± 0.22	1.29 ± 0.44	0.87 ± 0.05	92.4
	5	4.53 ± 0.51	3.85 ± 0.14	0.71 ± 0.06	0.48 ± 0.04	95.3
	9	26.97 ± 3.32	7.49 ± 0.16	1.06 ± 0.05	0.59 ± 0.11	93.7
	14	40.92 ± 9.36	15.56 ± 0.70	1.21 ± 0.04	0.70 ± 0.15	94.2
Cu	3	67.91 ± 14.74	1.57 ± 0.28	0.71 ± 0.05	0.00 ± 0.00	89.6
	5	1.62 ± 0.73	1.74 ± 0.85	0.67 ± 0.13	0.00 ± 0.00	79.3
	9	4.18 ± 2.06	4.34 ± 0.47	1.61 ± 0.22	0.00 ± 0.00	84.6
	14	5.44 ± 1.59	4.40 ± 0.40	0.58 ± 0.10	0.00 ± 0.00	93.9

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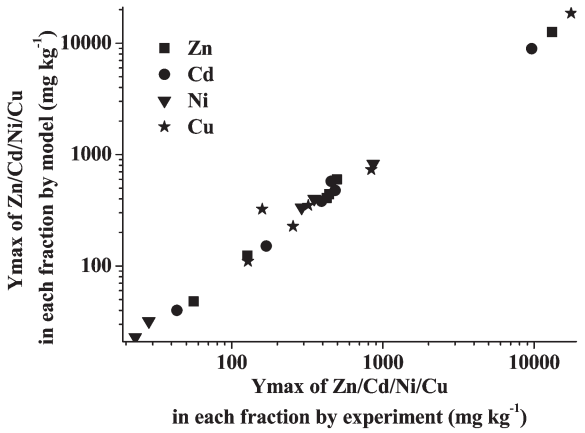

Fig. 2 Correlations between the predicted and actual maximum accumulation capacity y_{max} of Zn/Cd/Ni/Cu.

Discussion

Recent studies have shown that the exact subcellular distribution of metallic elements in plants may have significant consequences for phytoremediation due to their effects on detoxification and bio-accumulation.[26–30] As a plant species with potential phytoremediation ability, *Brassica juncea* is widely distributed in metal uncontaminated and contaminated areas of China.[10] In this study, we report the subcellular accumulation and distribution of metallic elements in Indian mustard and its kinetic application. The accumulation rate constant k and maximum accumulation capacity y_{max} were calculated to predict the accumulation and subsequent subcellular distribution of metals in plants.

During the 14-day metal exposure experiment, Indian mustard demonstrated potential phytoremediation ability for metal contamination (Fig. 2). Although numerous studies have investigated the relationship between metal uptake and accumulation in various organisms,[15–17] the use of kinetic models is a powerful tool to describe metal accumulation and the potential phytoremediation properties of selected species. In this study, the kinetic model adequately described the experimental data for accumulation by roots, stems, and leaves. The metal accumulation in most of the subcellular fractions of the roots and stems fitted the equation well. It was concluded that the kinetic model could be applied to predict the accumulation rate constant k and maximum accumulation capacity y_{max} of the plant and its subcellular fractions in hydroponics culture (Table 1).

The Indian mustard plants in the experiment had a healthy appearance, and suffered no mortality during the metal treatments over the time of exposure. However, from the 3rd day of exposure to the 14th day, the concentration of metallic elements was much higher in the roots than in the leaves and stems (Fig. 1), which indicates that some amounts of the metals were retained following long-distance translocation from the roots to the stems and leaves. Plant tolerance to metals can be achieved through a range of physiological and

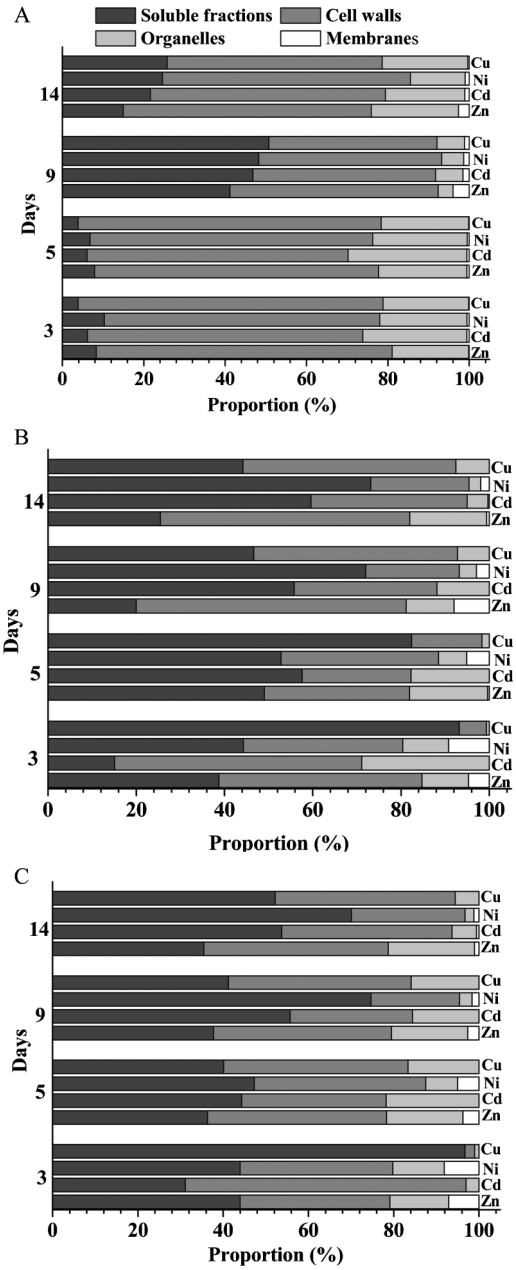

Fig. 3 Distribution of Zn, Cd, Ni, and Cu in water-soluble, cell wall, organelle, and cell membrane fractions of roots (A), stems (B), and leaves (C) as a function of time.

morphological changes. Metal retention by roots is often cited as a beneficial strategy. Zhang *et al.* hypothesize that there is a causal relationship between retention of Cu in the cell walls and uptake across the plasma membrane into the root cells, leading to a lower extent of transfer to the shoots.[31] Hall *et al.* proposed that the compartmentalisation of metallic elements into the epidermal cells is an important mechanism through which plants cope with high levels of metallic elements.[32]

Although metallic elements are retained following long-distance translocation from the roots to the stems and leaves, greater amounts of metallic elements accumulated in the

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stems and leaves of plants. However, only small amounts accumulated in the plant membranes (Fig. 3), even though these structures were sensitive to metallic elements. Excessive metal accumulation in plant membranes would interfere with cell activity and thus inhibit plant growth.[33,34] Metal tolerance and detoxification in plants might therefore depend on the subcellular distribution of the contaminants that are taken up. Plant cell walls are mainly composed of cellulose, xylan, and lignin. These compositions contain carboxyls, sulfhydryls, hydroxyls, amino groups, and aldehyde groups, which are the first barrier protecting the protoplasts from metal toxicity. The presence of metallic elements may result in a decrease in the free ion activity that is necessary to maintain normal physiological activities in plant cells.[35] Niu *et al.* proved that most of Cu was distributed mainly in the cell walls of maize.[36] Ramos *et al.* observed that the Cd in contaminated lettuce plants was largely present in the cell wall fraction,[6] and a similar subcellular distribution pattern has been reported in ramie.[7] In the present study, large amounts of Zn, Cd, Ni, and Cu were present in the cell wall fraction of the stems and leaves (Fig. 3), suggesting that the cell wall was the dominant site of metallic element storage in the stems and leaves. Once the metallic elements cross the cell wall, they are dissolved in the cytosol and partitioned into organelles. In this study, Zn, Cd, Ni, and Cu were found in the soluble fractions of the stems and leaves, suggesting that the soluble fraction serves as another storage compartment in both stems and leaves. The soluble cellular fraction, consisting mainly of the cytosol and largely concentrated in the cell matrix between cells or organelles, could be considered a buffering phase.[37] The vacuole is an important soluble organelle in the plant cell that occupies as much as 90% of the total cell volume in some cell types. Vacuoles not only store metallic elements but also contain organo-ligands, which are mainly sulfur-rich peptides, organic alkali compounds.[38] Küpper *et al.* found the vacuole of the leaf to be the major compartment of Ni sequestration in Brassicaceae, including *Thlaspi goesingense*.[39] Krämer *et al.* reported that the majority of leaf Ni in the hyperaccumulator *Thlaspi goesingense* was associated with the cell wall, and that the remaining Ni was localized in the vacuoles.[40] Harris *et al*. used a computer model for the speciation of the trace elements, which indicated that phloem sap is the nutrient rich fluid that transports sugars, amino acids and metal ions from leaves to other parts of the plant.[41] So the formation of complexes between metals and organo-ligands within the storage sites results in decreased free ion activity and thus reduced toxicity.[42,43]

Conclusion

Aqua-cultured seedlings of Indian mustard appear to have the potential be used as a cost-effective method for the removal of metals from contaminated waters. Therefore, we can draw a number of conclusions. First, the concentrations of Zn, Cd, and Ni in roots and stems increased with exposure time and then began to stabilise, whereas the metal concentrations in the leaves continued to rise between the 3rd and 14th day. Furthermore, the majority of metallic elements were associated with the cell wall, organelle, and soluble factions and only minor amounts of all metals were present in the cell membrane fraction. Finally, the kinetic model adequately described the experimental accumulation data of roots, stems, and leaves. Most of the subcellular fractions of the roots and stems fitted the equation well. The accumulation rate constant k and maximum accumulation capacity y_{max} of plant and subcellular fractions were calculated for the experiment. The results of this work enhance our understanding of metal transfer and transformation in plants and will be valuable for risk assessments of metal contamination at polluted sites.

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