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Removal of low concentrations of heavy-metal cation based on their fixation in plants

Xiao-Fang Pei†, Jun Wan†, Kun Zhang, Li-Hua Niu, Qian Du, Chun-Xue Zhang, Xin-Shi Wu, Bo Sun*

[a]*School of Chemistry and Chemical Engineering, Tianjin University of Technology, Tianjin 300384, China, email: pxfttkx@outlook.com (X.-F. Pei), junwantjlg@outlook.com (J. Wan), kunzhangtjlg@126.com (K. Zhang), lihuaniutjlg@163.com (L.-H. Niu), qdu2011tjlg@126.com (Q. Du), chxzhang@tjut.edu.cn (C.-X. Zhang), webwxs@126.com (X.-S. Wu), Tel. +86-22-60214259. Fax +86-22-23374289, email: sunbo_tjlg@hotmail.com (B. Sun)*

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ABSTRACT

Fixation of heavy metal cations in plant *Sedum spectabile* was studied. The key heavy-metal cation fixation species (ions or function groups) and the auxiliary species that promote heavy-metal cation fixation in the plant were investigated. The species involved in the fixation of tested heavy-metal cations (Cd^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+} and Zn^{2+}) were first surmised based on changes of the elemental composition in plant's roots. Their roles in the fixation of heavy metals were assessed and confirmed using simulated precipitation and adsorption of heavy-metal cations under near physiological conditions (20–35°C, pH 6–7.5). CO_3^{2-} and PO_4^{3-} were the key Cd^{2+}- fixing species, and Fe^{3+} ions played an auxiliary role in the precipitation of Cd^{2+} ions. PO_4^{3-}-containing species were also key species for the fixation of Cu^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}, and Ca^{2+} and HCO_3^- ions aided the precipitation of these cations. In the presence of a complexing agent, the pH of the solution was a key factor that can severely affect the removal of heavy metal cations. At pH 7–7.5 and in the presence of citrate, the removal percentage of Cu^{2+} from the solution can be as high as 99.0%, with residual concentration as low as 0.05 mg/L. Based on the obtained results, a biomimetic composite made of sodium alginate and calcium-containing phosphate was prepared via co-precipitation in the presence of Mg^{2+} and HCO_3^- ions under near-physiological conditions. The composite was excellent for removing low concentrations of heavy-metal cations under near-neutral conditions.

Keywords: Water treatment; Heavy-metal cations; Biomimetic separation; Biomimetic removal

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1. Introduction

Biological, chemical and physical methods are commonly used for removing heavy metals from aqueous solutions [1–3]. Biological methods, such as fungus and algae, have received great attention due to their environmental friendliness [4–11]. However, compared with chemical and physical methods, removal of heavy metals by biological methods seems to be less competitive. This is because heavy metals are not the biological composition of organisms, inferring that the enriched amount of heavy metals by organisms is limited and the theoretical uptake of heavy-metals in a biomass is small [9–11]. Nevertheless, organisms are sensitive to trace concentrations of heavy metals, and the growth of organisms can be seriously suppressed under heavy metal stress, even leading to death [12,13].

The efficiency of chemical and physical removal of heavy metals mainly relies on the components of the materials used [14–16]. Many heavy-metal-binding materials are effective entrapment agents when the concentrations of heavy-metal ions are high, while they are less effective when the concentrations of heavy-metal ions are low (<1 mg/L) [16–18]. This

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*Corresponding author.

†These authors contributed equally to this work and should be considered co-first authors.

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is because at low concentrations of heavy metals, the binding of heavy metals to the binding material is not strong enough, or the binding equilibrium concentrations of heavy metal ions are not low enough, which limits the amount of heavy metal ions that can be removed [19]. Therefore, it is necessary to develop an effective and economic method to remove low concentrations of heavy metals.

Heavy metals enter plants in a water-soluble form and are fixed in the plant's body [20,21]. In our study, we found that when *Sedum spectabile* [cf. Fig. S1 in Supporting Information (SI)] was cultivated in Cd^{2+}-containing culture medium, the root of the plant accumulated a considerable amount of Cd, but the leaves contained very low level of Cd (cf. Table S1 in SI), indicating that the root has the ability to fix Cd to prevent the spread of the heavy metal to other organs. Heavy metals exert their harmful actions through interactions with some chemical components in organisms [12,13,22–24]. Some species in organisms are sensitive to trace concentrations of heavy metals, and they may have the potential to fix heavy metals, most likely through a synergistic action among them [22]. Based on this observation, we explored the key species as well as auxiliary species that exhibited strong heavy metal cation fixation capacity in *Sedum spectabile* in an effort to provide useful information for effective and economic removal of low concentrations of heavy metal cations.

In this study, *Sedum spectabile* was cultivated in aqueous solution containing a heavy-metal cation to allow the enrichment of the heavy metal in plant's roots. The elements or species that were involved in the fixation of the heavy metal were first surmised via the changes of the elemental composition in the roots in the presence or absence of heavy metal cations. The roles of these species in the fixation of heavy metals were assessed and confirmed using simulated precipitation and adsorption of the heavy-metal cations under near physiological conditions (20–35°C, pH 6–7.5) using chemicals containing the surmised species.

Based on the results of the above experiments, a biomimetic composite containing the heavy-metal-fixing species was prepared and its heavy-metal fixation capacity was examined. The design of the biomimetic agent incorporated economic-effective removal of low concentrations of heavy-metal cations, environmental compatibility, and minimization of secondary pollution.

Notably, fixation of heavy metal cations by plant is under physiological conditions, which are near neutral conditions. For heavy-metal cation-containing acidic wastewater, it is more economic to remove heavy metal cations under near-neutral conditions than under conventional alkaline conditions.

2. Experimental section

2.1. Cultivation of plants in heavy-metal-containing aqueous solution

Since water-soluble heavy metals can be easily taken up by plants [20,21], hydroponics was used to accumulate heavy metals in plants.

Sedum spectabile (Fig. S1 in SI) was selected as the hydroponic plant in the present study. Four variants of Hoagland's nutrient solution with different nutrient deficiency, denoted as culture medium A, B, C and D, were used to culture the plant (cf. Table S2 in SI) [20–22]. The cultivation of plants in heavy metal-containing aqueous solution was similar to the method that was reported in our previous work [22], except that arsenic-containing anions were replaced by certain amounts of heavy-metal cations (Cd^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+} or Zn^{2+}). Heavy metals were provided as their nitrate compounds, that is, $Cd(NO_3)_2$, $Cu(NO_3)_2$, $Ni(NO_3)_2$, $Mn(NO_3)_2$, and $Zn(NO_3)_2$. When necessary, an appropriate amount of disodium ethylene diaminetetra acetate was added to the solution to prevent the precipitation of heavy-metal cations. Cultivations without heavy-metal ions were simultaneously carried out as controls.

2.2. Treatment and elemental analysis of plant roots after cultivation in heavy-metal-containing aqueous solution

The treatment and elemental analysis of plant roots after cultivation were carried out according to the method reported in the literature [22,25–29]. The general procedure was as follows:

The cultivated roots were thoroughly washed with deionized water and dried at 40°C in an oven under reduced pressure until constant weights of the roots were obtained. The dried roots were ground to fine powder and passed through a standard aperture sieve with a pore size of ca. 0.45 mm.

An accurately weighed powder sample (0.1 ± 0.001 g) was placed in a beaker, and 10 mL of concentrated HNO_3 was added to the sample. The mixture was kept at room temperature for 24 h and heated until it was nearly completely dissolved. Subsequently, another 10 mL of concentrated HNO_3 was added, and the sample was reheated. When obvious red-brown smoke appeared, 5 mL of $HClO_4$ was added into the beaker, and the heating process continued until the smoke disappeared and a transparent and colorless solution appeared.

The residues were diluted to 50 mL with deionized water in a calibrated volumetric flask, and the resulting solution was subjected to inductively coupled plasma optical emission spectrometry (ICP-OES) to determine the element contents in the roots. Control digestion without the sample was also conducted under the same conditions.

2.3. Simulated precipitation of heavy metals by surmised heavy-metal fixing species

The simulated precipitation of heavy metals was performed using a method similar to that reported in our previous work [22]. The general procedure was as follows: A certain amount of tested heavy-metal cations and the surmised heavy-metal-fixing species were added into a beaker containing 200 mL of deionized water at room temperature under vigorous agitation. The solution was stirred, and the pH was maintained within a range of 7–7.5 (close to the plant physiological conditions) with 0.1 mol/L HNO_3 or NaOH solution until no more precipitation was formed and the pH of the solution was stable. The mixture was kept still overnight, centrifuged and filtrated. The contents of the tested heavy metals and other elements in the supernatant were determined by ICP-OES.

2.4. Adsorption of heavy metals by water-insoluble chemicals containing surmised heavy-metal-fixing species

An accurately weighed adsorbent containing the surmised heavy-metal-fixing species (0.1 ± 0.001 g) was placed into a 200 mL beaker. Subsequently, 50 mL heavy-metal cation-containing solution was added to the beaker. The mixture was intermittently agitated, kept still at room temperature for 24 h, centrifuged and filtrated. The contents of heavy metals and other related elements in the solution were determined by ICP-OES.

2.5. Precipitation of Cu^{2+} from aqueous solutions in the presence of citrates

At room temperature and under vigorous agitation, a certain amount of $Ca(H_2PO_4)_2\cdot H_2O$ was added into 50 mL of a solution containing 5.0 mg/L Cu^{2+} and 151.2 mg/L citric acid (The molar ratio of citric acid: Cu^{2+} ions was 10:1). The solution was stirred, and the pH was adjusted within a range of 7–7.5 (close to the plant physiological conditions) with NaOH solution or $Ca(OH)_2$ powder. The mixture was kept still overnight, centrifuged and filtrated. Cu content in the supernatant was determined by ICP-OES.

2.6. Preparation of a biomimetic composite containing sodium alginate and calcium-containing phosphate in the presence of Mg^{2+} and HCO_3^- Ions

The general procedure was as follows:

A solution containing Ca^{2+} and Mg^{2+} ions was prepared by dissolving 0.638 g of anhydrous $CaCl_2$ (5.75 mmol Ca) and 0.233 g of $MgCl_2\cdot H_2O$ (1.15 mmol Mg) in 10 mL of water. Then 5 mL of 6 wt % H_3PO_4 (ca. 3.1 mmol P) solution and 0.021 g of $NaHCO_3$ (0.25 mmol C) in 5 mL of water were added into the Ca^{2+}-Mg^{2+} solution. A solution of sodium alginate was prepared by dissolving 0.2 g of sodium alginate powder in 50 mL water.

Under vigorous stirring, the above two solutions were added drop wise to a vessel containing 25 mL water. The temperature of the vessel was maintained at about 25°C and the pH of the mixture was controlled within 7.2–7.5 by adding 5 wt % aqueous NaOH. After all the solutions were added and no further change in pH was observed over time (about 5 h), the resulting solid was allowed to stand at room temperature overnight. Afterwards, the precipitate was isolated and washed several times with deionized water until the pH of the washing medium was neutral. The solid was dried at 40°C in vacuo to a constant weight. About 0.76 g of a light-yellowish material was obtained, with a molar ratio of Ca to P at 1.61.

2.7. Measurements

The concentrations of heavy-metal ions and other ions in an aqueous solution were determined with a Vista MPX ICP-OES Spectrometer (Varian, Inc., Palo Alto, CA, USA). (The operation conditions of the instrument are shown in Table S3 in SI).

The pH of the solutions was measured by a Model pHS-25 pH Meter (Shanghai Rex Instruments Factory, Shanghai, China) with combined glass electrode.

Powder X-ray diffraction (XRD) patterns were recorded on a Rigaku D/MAX-2000PC X-ray diffractometer, with Cu Kα radiation (λ = 0.154 nm) at 100 mA and 40 kV. A scan rate of 10°C/min and a step size of 0.02° were used over a 2θ range of 3–80°.

The content of carbon in the samples was determined using a vario-EL element analyzer (Elementar Analysen systeme GmbH). The precision of the results was estimated to be 0.3%.

X-ray photo electron spectroscopy (XPS) was performed with a Perkin Elmer PHI-1600 electron-spectroscopic–chemical analyzer (ESCA) (Wellesley, MA) equipped with a hemispherical electron energy analyzer and a Mg Kα monochromator source (light Quantum energy (hν) = 1253.6 eV). The pressure in the analysis chamber during data acquisition was ~10^{-7} Pa. The analysis area was 0.8 mm^2. The survey spectra were carried out with a pass-energy of 187.85 eV. The contaminant C1s peak at 284.6 eV was used to calibrate the energy shift. The accuracy of the binding energy is estimated to be ± 0.2 eV. All spectral data was treated with PHI Multipak software (version 6.0, Wellesley, MA).

The surface morphology of a sample was observed by a JEOL JSM-6700F field-emission scanning electron microscope (FE-SEM) (JEOL Ltd., Tokyo, Japan). The samples were mounted on a copper conductive tape and coated with Au to minimize sample charging.

3. Results and discussion

S. spectabile can survive in the aqueous solution containing cadmium (II), copper (II), nickel (II), manganese (II), or zinc (II), and exhibited an accumulation of the heavy metals (cf. Tables S4–S8 in the SI). Therefore, it was selected as the hydroponic plant for the study. In order to explore the key species as well as the auxiliary species for heavy-metal fixation in plant's roots, we designed four variants of Hoagland's nutrient solution (cf. Table S2 in SI) to compare the elemental composition of the roots under different cultivation conditions [*e.g.* under macro nutrient (P, S, Ca, or Mg)-deficient condition]. No micro nutrient was added in all four culture media, except when the plant was under the stress of manganese, copper or zinc. In parallel, the control cultivation (without heavy-metal stress) with similar growth status and similar ages to the treated ones was also conducted under the same ambient conditions. One control was used for each culture medium.

3.1. Investigation of the cadmium fixation in plant roots

3.1.1. Comparison of growth status of plants under different conditions and elemental analysis of the cadmium-fixing elements in plant roots

After 9-week cultivation under Cd^{2+}-stress, we observed that a considerable amount of Cd was fixed in tested plant roots (cf. Table S4 in SI), while only a small amount of Cd was detected in plant leaves (≤0.05 mg/g) (cf. Table S9 in SI). A possible explanation for this phenomenon is that when Cd^{2+} ions invaded roots of a plant, the plant would spontaneously fix these foreign ions in its root as soon as possible to prevent them from entering its important organs and tissues [20–22]. Therefore, some species of the plant that could be necessary for the normal growth of a plant might be involved in the cadmium fixation, leading to suppressed plant growth. Since the tested plants could still maintain a growth in the Cd^{2+}-containing solution (cf. Table S10 in SI), we inferred that the elements involved in the cadmium fixation were mainly macro nutrients [20,21].

In addition, a few leaves of plants cultivated under Cd^{2+} stress, especially those cultivated in culture medium D (without any nutrient), became chlorotic and even fell off the plant, suggesting that some micro nutrients may also be involved in the cadmium fixation [20,21]. No micro nutrient was added in all the culture media. The chlorotic symptom could be attributed to the loss of micro nutrients in leaves. We inferred that when Cd^{2+} ions entered plant's roots, the plant may spontaneously transport some micro nutrient-containing species from leaves to its roots to fix Cd^{2+} ions, causing nutrition loss in leaves.

Comparison of elemental contents of the roots between the Cd^{2+} stressed cultivation and the control cultivation (cf. Table S4 in SI) showed that when cadmium was accumulated in roots, the contents of phosphorus, calcium and magnesium (three macro nutrients) were obviously increased in the roots cultivated in all four types of culture media, suggesting that the species containing P, Ca and Mg could be involved in the fixation of Cd^{2+} ions.

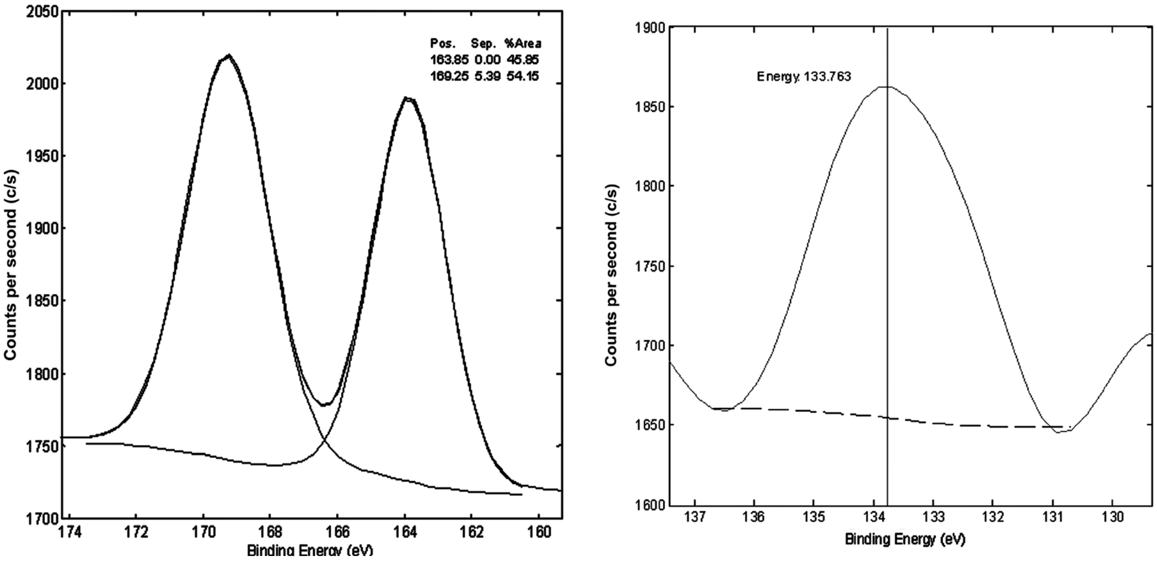
Besides, a slight increase of Fe content in roots was observed, implying that Fe (one of micro nutrients) was likely to be involved in cadmium fixation. This might be one of the reasons why some leaves became chlorotic under cadmium stress. The Fe deficiency that was initiated by transportation of Fe from plant's body to roots for the fixation of invaded cadmium may cause leaves to become chlorotic [30–33].

The contents of other micro nutrients, such as Cu, Zn, Mo, Ni, Mn, and Co were very low in roots (not listed in the Table S4 in SI), suggesting that they were unlikely to be involved in cadmium fixation.

In order to obtain more information about cadmium-fixing species, we also investigated the chemical bonding state of Cd^{2+} ions in plant's roots cultivated with and without Cd^{2+} stress (Figs. 1 and 2). After cultivation under Cd^{2+} stress, the $Cd(3d_{5/2})$ peak was separated into two Gaussian peaks centered at 405.13 and 406.00 eV (Fig. 2a). These values were close to the binding energy of Cd atoms binding with O atoms, and thus they could be assigned to the Cd atoms fixed in the form of $CdCO_3$, $Cd_3(PO_4)_2$ or $Cd(OH)_2$ (http://www.lasurface.com/database/elementxps.php). The $S(2p_{3/2})$ spectrum of plant's roots of control cultivation exhibited two peaks centered at 163.85 and 169.25 eV (Fig. 1a). The former peak could be assigned to S atoms in form of SO_4^{2-}, and the latter one could be attributed to the S atoms in reducing state such as S^{2-}, CH_3S-, and -SH. After cultivation under Cd^{2+} stress, the peak around 163.85 eV was separated into two Gaussian peaks centered at 161.24 and 163.48 eV (Fig. 2b). The one at 161.24 eV could be from the S atoms that were binding with Cd atoms. Because there were no obvious changes of the chemical bonding state of P atoms in K_2HPO_4/KH_2PO_4 and $Cd_3(PO_4)_2$, no remarkable difference was observed between the two $P(2p_{3/2})$ spectra in plant's roots cultivated with and without Cd^{2+} stress (Fig. 1b and Fig. 2c).

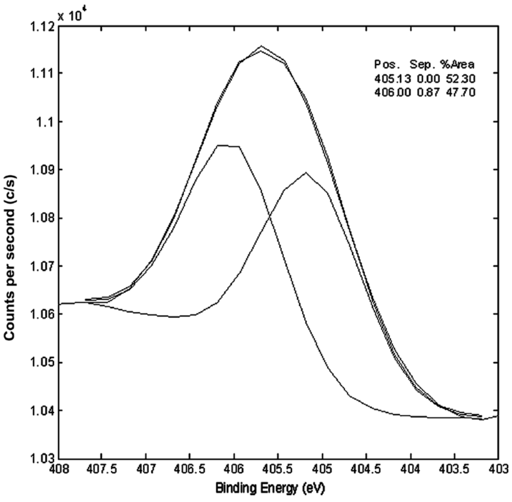
3.1.2. Simulated precipitation of Cd^{2+} ions to confirm the cadmium-fixing species

It is generally believed that heavy metals enter plants in a water-soluble form, and then are fixed in the plant's body [20,21]. To confirm the exact species involved in cadmium fixation, we performed simulated precipitation of

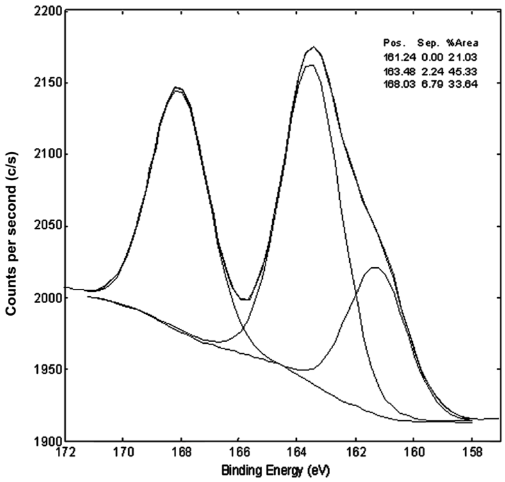


(a) $S(2p_{3/2})$ (b) $P(2p_{3/2})$

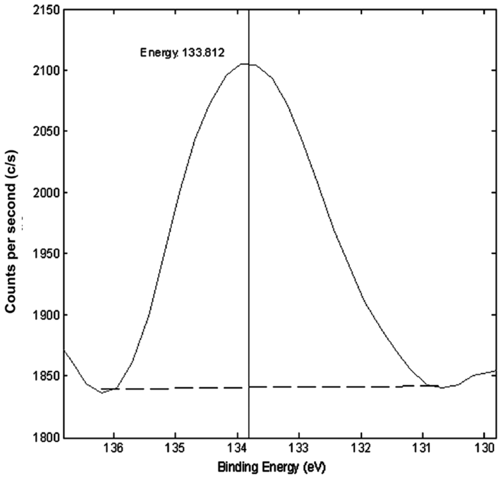
Fig. 1. XPS spectra of plant roots after cultivation without heavy-metal stress: (a) $S(2p_{3/2})$ (b) $P(2p_{3/2})$.



(a) Cd($3d_{5/2}$)



(b) S($2p_{3/2}$)



(c) P($2p_{3/2}$)

Fig. 2. XPS spectra of plant roots after cultivation under Cd^{2+} stress (a) Cd($3d_{5/2}$) (b) S($2p_{3/2}$) (c) P($2p_{3/2}$).

Cd^{2+} ions by adding the surmised species containing P, Ca, and Fe into the Cd^{2+}-containing aqueous solution. The S- and C-containing inorganic species (elements S and C also belong to macro nutrients) [20,21,34], such as HS^-/S^{2-} and HCO_3^-/CO_3^{2-}, were also evaluated.

Cd^{2+} ions were completely precipitated to form $Cd(OH)_2$ only in an aqueous alkaline solution (cf. Table S11 in SI). In a near-neutral solution, HCO_3^-/CO_3^{2-}- or PO_4^{3-}-containing species were strong cadmium-fixing species. This could be explained by the solubility product constant [35]. However, the solubility product constants of cadmium-containing phosphates such as cadmium phosphate ($Ksp = 2.53\times10^{-33}$) are much higher than those of the corresponding lead-containing phosphates such as lead phosphate ($Ksp = 8.0\times10^{-43}$). Therefore, compared with lead fixation, fixation of cadmium by PO_4^{3-}-containing species was weaker [36]. Similar to its function in lead fixation, Ca^{2+} ions played an auxiliary role in cadmium fixation by PO_4^{3-}-containing species.

The combination of HCO_3^- and Fe^{3+} exhibited a strong Cd^{2+} ion fixation effect, leading to near complete precipitation of Cd^{2+} ions (cf. Table S11 in SI). This enhanced fixation effect caused by both Fe^{3+} and HCO_3^- could be explained as follows: Fe^{3+} ions were easily hydrolyzed to form ferric hydroxide, which adsorbed acidic HCO_3^- ions on its surface and formed a primary adsorbing layer to attract Cd^{2+} ions as the counter ion, thus promoting the precipitation of $CdCO_3$ [37].

Elemental analysis of the cadmium-containing precipitate collected in the presence of HCO_3^- and Fe^{3+} ions showed that the molar ratio of Cd:C:Fe was 1.4:1.5:1. Fig. 3 shows the XRD pattern of the precipitate. The peaks at 2θ = 23.44°, 30.30°, 36.64°, 43.80°, 49.92°, 58.98°, 61.74, 58.98° are charac-

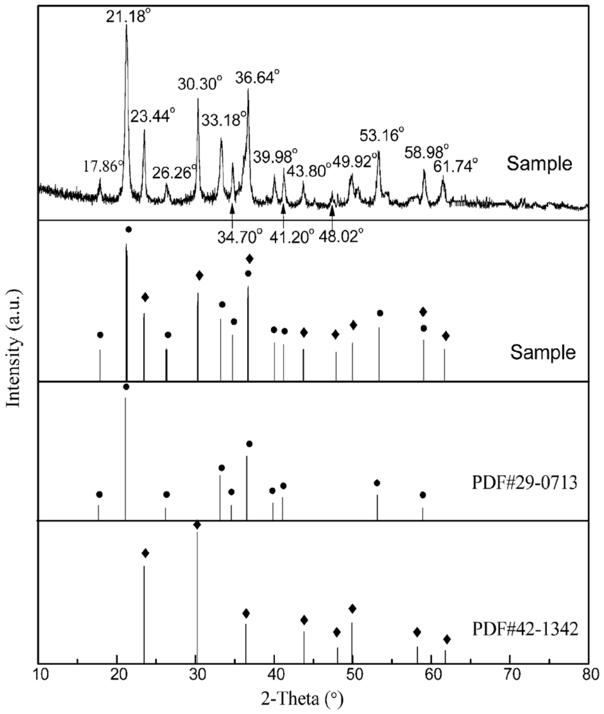


Fig. 3. XRD pattern of the collected cadmium-containing precipitate in the presence of $Fe^{3+}$and HCO_3^-

teristic peaks for $CdCO_3$ (PDF#42-1342) and the peaks at 2θ = 17.86°, 21.18°, 26.26°, 33.18°, 34.70°, 36.64°, 39.98°, 41.20°, 53.16° and 58.98° are for FeO(OH) (PDF#29-0713).

3.1.3. Adsorption of Cd^{2+} ions by the chemicals containing the cadmium-fixing species

The adsorption of Cd^{2+} ions by some water-insoluble chemicals was compared to further confirm the key species with the strongest cadmium fixation capacity. Calcium oxalate was also included to compare the cadmium fixation capacity between carboxyl groups that are strong organic cadmium fixation species [16,18] and inorganic species. Table 1 reveals that carbonates and phosphates had the strongest cadmium fixation capacity.

The above results confirmed that CO_3^{2-}- and PO_4^{3-}-containing species were the key species for cadmium fixation, whereas Fe^{3+} and Ca^{2+} ions were the auxiliary species to facilitate the fixation. Cadmium fixation was almost complete through the formation of cadmium-containing carbonate or phosphate, and the presence of Fe^{3+} or Ca^{2+} could accelerate the fixation process.

3.2. Investigation of the fixation of Cu^{2+}, Ni^{2+}, Mn^{2+} or Zn^{2+} ions in plant roots

By similar cultivation, elemental analysis of cultivated roots, simulated precipitation and adsorption analysis, we confirmed that the roots were main sites for the fixation of Cu^{2+}, Ni^{2+}, Mn^{2+} and Zn^{2+} cations, and PO_4^{3-}-containing species were also the key species for the fixation of these cations (*cf*. Tables S5–S8, S12–S19 in SI). Table 2 compares PO_4^{3-}-containing chemicals and other agents containing groups that are likely to bind Cu^{2+}, Ni^{2+}, Mn^{2+} and Zn^{2+} cations.

3.3. Investigation of the role of Mg^{2+} ions

It should be noted that after cultivation under the stress of Cu^{2+}, Ni^{2+}, Mn^{2+}, or Zn^{2+} cations, the contents of magnesium, calcium and phosphorus were simultaneously increased in roots. However, the adsorption of the tested heavy-metal cations on magnesium-containing phosphate is not as favorable as on calcium phosphate. In order to explain the increase of magnesium, we compared the precipitation of calcium-containing phosphate in the presence and absence of Mg^{2+} ions. The composition of solution used in the study was referred to Hoagland's nutrient solution containing 5 mmol/L Ca, 1 mmol/L P, and 2 mmol/L Mg [20,21]. The results showed that the presence of Mg^{2+} can raise the pH of the solution where the precipitation of calcium-containing phosphate occurs, thus impeding the formation of calcium hydrogen phosphate and hydroxyapatite, which is partially confirmed by the increased ratio of Ca to P in the collected precipitate. The molar ratio of Ca to P was 1.39 when Mg was absent, while it was 1.41 when Mg was present, and the molar ratio of Ca plus Mg to P was about 1.55:1. Hydroxyapatite has the lowest solubility in water among calcium-containing phosphates and is less effective in the removal of heavy metal ions [36]. Besides, the calcium phosphate-containing precipitate with Mg^{2+} ions (The molar ratio of Ca to Mg is about 8:1) exhibited a similar XRD pattern (Fig. 4a) to that of calcium-containing phosphate precipitate without Mg^{2+} (Fig. 4b), with characteristic peaks at 2θ = 25.88° and 32.12°, respectively. However, the former had larger peak-widths at half height, indicating that it has poor crystallinity and smaller crystal grains than the latter. This coincides with the result reported in the literature [38]. We concluded that one reason for increased content of magnesium in plant's roots after the cultivation under heavy-metal stress is that magnesium co-precipitates or mixes with calcium-containing phosphate to lower the latter's crystallinity.

Table 1
Adsorption of Cd^{2+} by adsorbents containing surmised cadmium-fixing species[a]

Adsorbents[b]	Final concentration of Cd^{2+} in solution (mg/L)	Cd^{2+}-removed (%)
Calcium carbonate	≤0.01	≥99.8
Calcium phosphate	0.04	99.2
Calcium oxalate	3.74	25.2
Iron (III) hydroxide	1.32	73.6

[a] Amount of adsorbents = 0.1 g, initial concentration of Cd^{2+} in solution = 5.0 mg/L, total volume of solution = 50 mL, pH = 6–7, exchange time = 24 h, temperature = 25°C; [b] The grain-size was about 65–74 µm.

3.4. Removal of heavy-metal cations from aqueous solutions in the presence of a complexing agent

There are a variety of water-soluble complexing agents in plants, such as citrates, tartrates, malates, and the like.

Table 2
Adsorption of Cu^{2+}, Ni^{2+}, Mn^{2+} and Zn^{2+} by PO_4^{3-}-containing chemicals and other reagents[a]

Adsorbents[b]	Metal cations	Final concentration of M^{2+} in solution (mg/L)	M^{2+}-removed (%)
Calcium phosphate	Cu^{2+}	≤0.01	≥99.8
	Ni^{2+}	0.08	98.4
	Mn^{2+}	0.06	98.8
	Zn^{2+}	0.06	98.8
Calcium carbonate	Cu^{2+}	0.02	99.6
	Ni^{2+}	1.46	70.8
	Mn^{2+}	0.08	98.6
	Zn^{2+}	0.19	96.2
Iron(II) sulfide	Cu^{2+}	0.23	95.4
	Ni^{2+}	2.08	58.4
	Mn^{2+}	4.38	12.4
	Zn^{2+}	3.37	32.6

[a] Initial concentration of M^{2+} in solution = 5.0 mg/L, M^{2+} = Cu^{2+}, Ni^{2+}, Mn^{2+}, or Zn^{2+}, amount of adsorbents = 0.1 g, total volume of solution = 50 mL, pH = 6–7, exchange time = 24 h, temperature = 25°C; [b] The grain-size was about 65–74 µm.

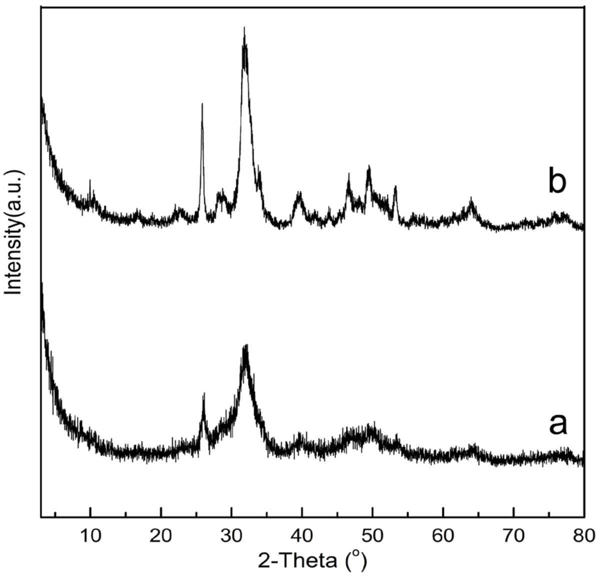


Fig. 4. XRD patterns of the collected calcium-containing phosphate precipitates (a) with Mg^{2+} and (b) without Mg^{2+}.

Table 3
Effect of pH on the removal of Cu^{2+} in the presence of citrate^a

pH of solution	Final concentration of Cu^{2+} in solution (mg/L)	Cu^{2+}-removed (%)
6–6.5	0.70	86.0
6.5–7.0	0.61	87.8
7.0–7.5	0.12	97.5
7.5–8.0	0.14	97.2
8.0–8.5	1.45	71.0
ca. 7.0^b	0.05	99.0

^{a}Initial concentration of Cu^{2+} in solution = 5.0 mg/L, molar ratio of citric acid to Cu^{2+} = 10:1, amount of $Ca(H_2PO_4)_2{\cdot}H_2O$ = 0.1 g, total volume of solution = 50 mL, exchange time = 24 h, temperature = 20°C. pH was adjusted with NaOH; ^{b}pH was adjusted with 40 mg of $Ca(OH)_2$ instead of NaOH.

The fixation of heavy metal cations by calcium phosphates can be severely influenced by complexing agents (*cf*. Tables S20 in SI). The pH can affect both the stability of the complex and the precipitation of phosphates. Generally, as pH increases, more heavy-metal cations could be tightly held by the complexing agent, which adversely affects their removal by precipitation; as pH decreases, the concentration of PO_4^{3-} species decreases, which also adversely affects the precipitation of heavy-metal cations as phosphates [37].

Cu^{2+} ions usually form stronger coordination bonds with many complexing agents than Cd^{2+}, Ni^{2+}, Mn^{2+} or Zn^{2+} ions [37]. Citrate is one of the strongest complexing agents among organic acids commonly existing in plants (*cf*. Tables S21 in SI). Thus, taking the removal of low concentration of Cu^{2+} as an example, the effect of pH on the removal of Cu^{2+} in the presence of citrate (Table 3) was investigated.

As shown in Table 3, pH is surely a key factor for the removal of Cu^{2+} ions in the presence of a complexing agent. At an appropriate pH, the percentage of Cu^{2+} that were removed in the presence of a citrate could be as high as 99.0% and the residual Cu^{2+} concentration could be as low as 0.05 mg/L.

3.5. Removal of heavy-metal cations from aqueous solutions by the biomimetic composite

Some aquatic plants such as algae have been demonstrated to be effective biosorbents for the removal of heavy metals [39–41]. However, their heavy-metal-binding capacity (or their theoretical uptake per gram of mass) is obviously lower than that of synthetic sorbents. This is because heavy metals are not the biological components of organisms. Hence the fixed amount of heavy metals by organisms is very limited.

Based on the our results, we designed a biomimetic synthetic sorbent (a composite) made of sodium alginate, a complexing agent in alga (a sea-plant), and calcium-containing phosphate. The composite was prepared in the presence of Mg^{2+} and HCO_3^- ions under near-physiological conditions of plants (25°C, pH 7.2–7.5). The obtained biomimetic composite was expected to possess several heavy-metal-fixing groups, a large surface area, and good hydrophilicity and permeability.

The FT-IR spectrum (Fig. 5) of the composite prepared under the conditions given in the Experimental Section showed a strong, broad band at 3423.1 cm^{-1} attributed to O-H stretching vibration, a band at 1635.4 cm^{-1} attributed to C=O asymmetric stretching vibration, two bands at 1430.9 cm^{-1} and 1299.8 cm^{-1} assigned to coupled C-O stretching vibration and O-H deformation vibration of carboxyl group, and a strong band at 1062.6 cm^{-1} and a band at 557.3 cm^{-1} assigned to PO_4^{3-} stretching and bending vibration [42,43], suggesting that a composite material containing alginate and phosphate was formed.

The surface morphology of the composite revealed that the composite is composed of nano-size particles with irregular shapes and little crystalline (Fig. 6). The size of the particles was mainly in the range of 20–30 nm. Some particles were smaller than 20 nm. The biomimetic sorbent had no apparent water absorbency, which was distinctly different from alginate hydrogel. This means that the composite has no apparent swelling behavior in aqueous solution, which makes it a potential bedding material. In addition, it possesses several heavy-metal-fixing groups, including carboxyl group and calcium-carbonate-containing phosphate. Thus, it is a more effective heavy-metal fixing agent compared with those with only one type of function group.

Taking the removal of low concentration of Ni^{2+} ions as an example (compared with other cations, its removal efficacy is low, as shown in Table 4), we found that the composite exhibits more effective Ni^{2+} removal than those with only one type of function group (Table 4).

PO_4^{3-} ions are more effective Ni^{2+} ion fixation species than HPO_4^{2-} (The solubility product (Ksp) of $Ni_3(PO_4)_2$ is 4.74×10^{-32} [35]). The synergistic effects of several species or groups in the composite can be explained as follows: similar to the role of Mg^{2+} ions (*cf*. Section "Investigation of the Role of Mg^{2+} Ions), the presence of HCO_3^- ions can also prevent the formation of hydroxyapatite [36], lower the crys-

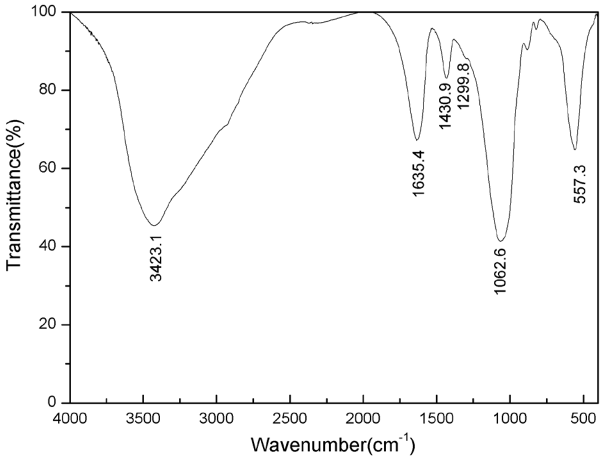


Fig. 5. FT-IR spectrum of the prepared biomimetic composite containing sodium alginate and calcium-containing phosphate.

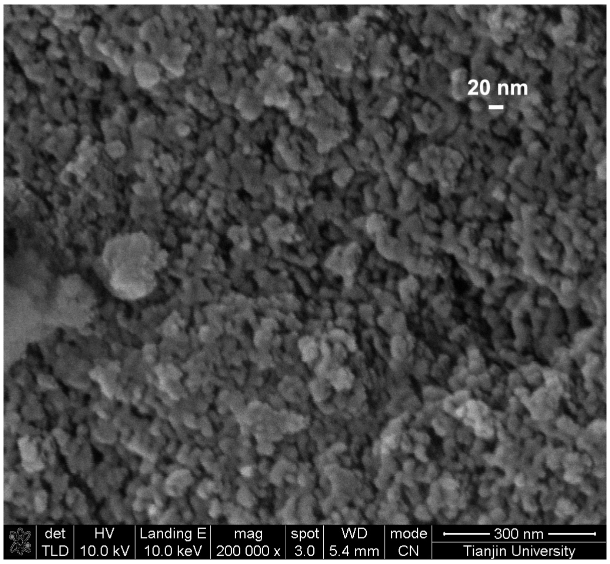


Fig. 6. SEM photomicrograph of the composite.

tallinity of calcium-containing phosphate, and thus enlarge the surface area of the material. Sodium alginate contains some carboxyl groups that are strong heavy-metal-binding groups. These carboxyl groups also provide the composite good dispersity, hydrophilicity and permeability. Furthermore, sodium alginate, which is a polyprotic base, or an H$^+$ acceptor, can serve as a buffer substance and help HPO_4^{2-}, which is the main form of phosphates in the composite under near-neutral conditions [37], to deprotonate smoothly.

4. Conclusions

In the present study, we found that the species containing CO_3^{2-} and PO_4^{3-} were the key Cd^{2+}-fixing species, and Fe^{3+} ions played an auxiliary role in the precipitation of Cd^{2+} ions. The PO_4^{3-}-containing species were also key species for the fixation of Cu^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}, and Ca^{2+} and HCO_3^- ions aided the precipitation of these cations. In the presence of a complexing agent, pH can severely affect the removal of heavy metal cations. At pH 7–7.5 and in the presence of a citrate, 99.0% Cu^{2+} can be removed from the solution, with the residual Cu^{2+} concentration as low as 0.05 mg/L. Based on these results, we prepared a biomimetic composite containing sodium alginate and calcium-containing phosphate via co-precipitation in the presence of Mg^{2+} and HCO_3^- ions under near-physiological conditions of plants. The composite was excellent for removing low concentrations of heavy-metal cations under near-neutral conditions.

Table 4
Comparison of Ni^{2+} removal capacity between the composite and related adsorbents[a]

Adsorbents[b]	Final concentration of Ni^{2+} in solution (mg/L)	Ni^{2+}-removed (%)
Composite	0.06	98.8
Calcium-magnesium-carbonate-containing phosphate[c]	0.10	98.0
Cross linked sodium alginate by calcium	0.97	80.6

[a] Amount of adsorbents = 0.1 g, initial concentration of Ni^{2+} in solution = 5.0 mg/L, total volume of solution = 50 mL, pH = 6–7, exchange time = 24 h, temperature = 25°C; [b] The grain-size was about 65–74 μm; [c] It was obtained by a method similar to that for the preparation of the composite, except that no sodium alginate was added.

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Associated Content

Supporting Information

Fig. S1 shows the photograph of *Sedum Spectabile*. Table S1 shows the comparison of Cd contents in roots and leaves of *Sedum Spectabile* cultivated under Cd^{2+} stress. Table S2 shows the formulations of four culture media in hydroponic culture. Table S3 shows the instrumental parameters and operating conditions for ICP-OES. Table S4 shows the comparison of elemental contents in the roots of *Sedum Spectabile* cultivated under Cd^{2+} stress. Table S5 compares the elemental contents in the roots of *Sedum Spectabile* cultivated under Cu^{2+} stress. Table S6 compares the elemental contents in the roots of *Sedum Spectabile* cultivated under Ni^{2+} stress. Table S7 compares the elemental contents in the roots of *Sedum Spectabile* cultivated under Mn^{2+} stress. Table S8 compares the elemental contents in the roots of *Sedum Spectabile* cultivated under Zn^{2+} stress. Table S9 compares the Cd contents in the leaves of *Sedum Spectabile* cultivated under Cd^{2+} stress. Table S10 compares the growth status of *sedum spectabile* with and without Cd^{2+} stress. Table S11 shows the results of simulated precipitation of cadmium in plant's roots. Table S12 compares the growth status of *sedum spectabile* with and without Cu^{2+} stress. Table S13 compares the Cu contents in leaves of *sedum spectabile* cultivated under Cu^{2+} stress, Table S14 compares the growth status of *sedum spectabile* with and without Ni^{2+} stress. Table S15 compares the Ni contents in leaves of *sedum spectabile* cultivated under Ni^{2+} stress. Table S16 compares the growth status of *sedum spectabile* with and without Mn^{2+} stress. Table S17 compares the Mn contents in leaves of *sedum spectabile* cultivated under Mn^{2+} stress. Table S18 compares the growth status of *sedum spectabile* with and without Zn^{2+} stress. Table S19 compares the Zn contents in leaves of *sedum spectabile* cultivated under Zn^{2+} stress. Table S20 compares the Cu^{2+} adsorption in the presence of citrate. Table S21 compares some organic-acids commonly existing in plants in terms of dissolution of copper phosphate or nickel phosphate.

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Supporting_Information

Fig. S1. *Sedum spectabile* for our experiment

Table S1
Comparison of Cd contents in roots and leaves of *Sedum Spectabile* cultivated under Cd^{2+} stress[a]

Duration of the cultivation (week)	Cd content in root cultivated under Cd^{2+} stress (mg/g)	Cd content in leaves cultivated under Cd^{2+} stress (mg/g)
9	23.70	≤0.05

[a] *S. spectabile* was cultivated in culture medium A (see below), the duration of the cultivation is 9 week (without Cd^{2+} stress in the first week), 15–25°C, total volume of culture solution is 200 mL. Every 7 days, about 10 mg of Pb^{2+} ions was added into the culture medium, and totally 80 mg of Pb^{2+} ions was added.

Culture medium	$Ca(NO_3)_2$ (mg/L)	KNO_3 (mg/L)	$NH_4H_2PO_4$ (mg/L)	$MgSO_4$ (mg/L)	NH_4Cl (mg/L)	$MgCl_2$ (mg/L)
A	820	607	115	241	–	–

Table S2
Formulations of four culture media in hydroponic culture

Cultural Medium	$Ca(NO_3)_2$ (mg/L)	KNO_3 (mg/L)	$NH_4H_2PO_4$ (mg/L)	$MgSO_4$ (mg/L)	NH_4Cl (mg/L)	$MgCl_2$ (mg/L)
A	820	607	115	241	–	–
B	820	607	–	241	93	–
C	820	607	115	–	–	190
D	–	–	–	–	–	–

Table S3
Instrumental parameters and operating conditions for ICP-OES

Radio frequency (R.F.) power	1.2 kW
Plasma gas flow rate	15 L/min
Flow rate of Argon auxiliary	1.5 L/min
Nebulizer gas flow rate	0.9 L/min
Precision (general)	1–3%
Ca analytical line (λ)	396.8 nm (detection limit 0.003 mg/L)
Mg analytical line (λ)	279.6 nm (detection limit 0.02 mg/L)
P analytical line (λ)	213.6 nm (detection limit 0.1 mg/L)
Cd analytical line (λ)	226.5 nm (detection limit 0.003 mg/L)
Cu analytical line (λ)	324.75 nm (detection limit 0.005 mg/L)
Mn analytical line (λ)	257.61 nm (detection limit 0.005 mg/L)
Zn analytical line (λ)	213.9 nm (detection limit 0.005 mg/L)
Ni analytical line (λ)	231.60 nm (detection limit 0.01 mg/L)
Fe analytical line (λ)	259.94 nm (detection limit 0.002 mg/L)
Mo analytical line (λ)	202.03 nm (detection limit 0.004 mg/L)
Co analytical line (λ)	228.62 nm (detection limit 0.005 mg/L)

Table S4
Comparison of elemental contents in the roots of *Sedum Spectabile* cultivated under Cd^{2+} stress[a]

Elements[b]	Contents of elements in *Sedum Spectabile*'s roots cultivated under Cu^{2+} stress (mg/g)[c]				Contents of elements in *Sedum Spectabile*'s roots cultivated without Cu^{2+} stress (mg/g)			
	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D
Cd	23.70	8.03	5.51	14.19	0.25	0.06	0.04	0.10
Ca	21.85	19.76	13.22	14.42	14.58	10.55	10.96	10.40
Mg	9.45	5.27	4.22	3.59	5.86	3.98	2.68	3.07
P	6.04	2.71	2.04	2.41	5.21	2.64	1.27	2.23
Fe	2.53	1.19	1.02	1.39	≤0.01	≤0.01	≤0.01	≤0.01

[a] The duration of the cultivation is 9 weeks, 25–35 °C, and total volume of culture solution is 200 mL.
[b] The contents of Zn, Cu, Mo, Ni, Mn and Co, etc, in roots were comparatively low, and not listed in the table.
[c] Every 7 days, 10 mg of Cd^{2+} ions was added into the culture medium, and totally 80 mg of Cd^{2+} ions was added.

Table S5
Comparison of elemental contents in the roots of *Sedum Spectabile* cultivated under Cu^{2+} stress[a]

Elements[b]	Contents of elements in *Sedum Spectabile*'s roots cultivated under Cu^{2+} stress (mg/g)[c]				Contents of elements in *Sedum Spectabile*'s roots cultivated without Cu^{2+} stress (mg/g)			
	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D
Cu	3.60	2.40	4.86	4.71	0.08	0.05	0.04	0.04
Ca	11.89	8.74	15.16	12.64	10.76	9.34	10.56	6.19
Mg	4.00	2.34	5.39	3.08	2.99	2.64	2.90	2.15
P	4.98	2.96	6.32	4.52	4.77	3.78	4.96	3.78
Fe	0.61	0.82	0.93	0.86	1.43	1.35	1.07	1.31
Mn	0.08	0.02	0.04	0.08	0.05	0.03	0.03	0.05
Ni	0.01	0.03	0.04	0.06	0.37	0.40	0.07	0.09

[a] The duration of the cultivation is 9 weeks, 25–35°C, and total volume of culture solution is 200 mL.
[b] The contents of Zn, Mo, Co, etc, in roots were comparatively low, and not listed in the table.
[c] Every one week, 20 mg of Cu^{2+} ions was added into the culture medium, and totally 160 mg of Cu^{2+} ions was added.

Table S6
Comparison of elemental contents in the roots of *Sedum Spectabile* cultivated under Ni^{2+} stress[a]

Elements[b]	Contents of elements in *Sedum Spectabile*'s roots cultivated under Ni^{2+} stress (mg/g)[c]				Contents of elements in *Sedum Spectabile*'s roots cultivated without Ni^{2+} stress (mg/g)			
	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D
Ni	21.46	6.14	8.39	10.35	0.37	0.40	0.07	0.09
Ca	23.29	19.97	25.22	11.05	10.76	9.34	10.56	6.19
Mg	6.02	4.06	5.75	2.39	2.99	2.64	2.90	2.15
P	8.21	6.91	6.17	3.87	4.77	3.78	4.96	3.78
Fe	1.28	1.44	1.09	0.85	1.43	1.35	1.07	1.31
Mn	0.07	0.09	0.03	0.03	0.05	0.03	0.03	0.05
Cu	0.05	0.03	0.05	0.04	0.08	0.05	0.04	0.04

[a] The duration of the cultivation is 9 weeks, 25–35 25–35°C, and total volume of culture solution is 200 mL.
[b] The contents of Zn, Mo, Co, etc, in roots were comparatively low, and not listed in the table.
[c] Every one week, 20 mg of Ni^{2+} ions was added into the culture medium, and totally 160 mg of Ni^{2+} ions was added.

Table S7
Comparison of elemental contents in the roots of *Sedum Spectabile* cultivated under Mn^{2+} stress [a]

Elements [b]	Contents of elements in *Sedum Spectabile*'s roots cultivated under Mn^{2+} stress (mg/g) [c]				Contents of elements in *Sedum Spectabile*'s roots cultivatedd without Mn^{2+} stress (mg/g)			
	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D
Mn	4.01	7.00	4.54	4.89	0.05	0.03	0.03	0.05
Ca	15.07	16.93	13.50	13.37	10.76	9.34	10.56	6.19
Mg	2.86	4.02	3.05	2.83	2.99	2.64	2.90	2.15
P	5.26	4.90	5.44	5.05	4.77	3.78	4.96	3.78
Fe	1.14	1.06	0.91	0.69	1.43	1.35	1.07	1.31
Cu	0.10	0.08	0.06	0.06	0.08	0.05	0.04	0.04

[a] The duration of the cultivation is 9 weeks, 25–35°C, and total volume of culture solution is 200 mL.
[b] The contents of Zn, Ni, Mo, Co, etc, in roots were comparatively low, and not listed in the table.
[c] Every one week, 20 mg of Mn^{2+} ions was added into the culture medium, and totally 160 mg of Mn^{2+} ions was added.

Table S8
Comparison of elemental contents in the roots of *Sedum Spectabile* cultivated under Zn^{2+} stress[a]

Elements[b]	Contents of elements in *Sedum Spectabile*'s roots cultivated under Zn^{2+} stress (mg/g)[c]				Contents of elements in *Sedum Spectabile*'s roots cultivated without Zn^{2+} stress (mg/g)			
	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D
Zn	6.23	1.93	3.33	2.34	≤0.02	≤0.02	≤0.02	≤0.02
Ca	11.54	11.68	15.25	14.60	10.76	9.34	10.56	6.19
Mg	3.30	3.20	3.94	3.08	2.99	2.64	2.90	2.15
P	5.58	4.65	5.08	5.17	4.77	3.78	4.96	3.78
Fe	0.91	0.77	0.67	0.73	1.43	1.35	1.07	1.31
Mn	0.03	0.09	0.05	0.02	0.05	0.03	0.03	0.05
Cu	0.07	0.01	0.04	0.05	0.08	0.05	0.04	0.04

[a] The duration of the cultivation is 9 weeks, 15–25°C, and total volume of culture solution is 200 mL.
[b] The contents of Ni, Mo, Co, etc, in roots were comparatively low, and not listed in the table.
[c] Every one week, 20 mg of Zn^{2+} ions was added into the culture medium, and totally 160 mg of Zn^{2+} ions was added.

Table S9
Comparison of Cd contents in the leaves of *Sedum Spectabile* cultivated under Cd^{2+} stress[a]

Elements	Contents of elements in *Sedum Spectabile's* leaves cultivated under Cd^{2+} stress (mg/g) [b]				Contents of elements in *Sedum Spectabile's* leaves cultivated without Cd^{2+} stress (mg/g)			
	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D
Cd	≤0.05	≤0.02	≤0.02	≤0.05	≤0.02	≤0.02	≤0.02	≤0.02

[a] The duration of the cultivation is 9 weeks, 25–35°C, and total volume of culture solution is 200 mL.
[b] Every 7 days, 10 mg of Cd^{2+} ions was added into the culture medium, and totally 80 mg of Cd^{2+} ions was added.

Table S10
Comparison of growth status of *Sedum Spectabile* with and without Cd^{2+} stress[a]

Cultural environment	Culture medium	Height of the plant before cultivation (cm)	Height of the plant after cultivation (cm)
With Cd^{2+} stress[b]	A	40	44
	B	38	43
	C	41	43
	D	38	42
Without Cd^{2+} stress	A	27	31
	B	30	33
	C	32	35
	D	30	33

[a] The duration of the cultivation is 9 weeks (without Cd^{2+} stress in the first week), and total volume of culture solution is 200 mL.
[b] Every 7 days, about 20 mg of Cd^{2+} ions was added into the culture medium, 25–35°C, and totally 160 mg of Cd^{2+} ions was added.

Table S11
Results of simulated precipitation of cadmium in plant's roots

Species in simulated system [b]	Molar ratio of Cd to other species (mol/mol)	Final concentration of elements in solution (mg/L)				Cd^{2+}-precipitated (%)
		Cd	P	Fe	Ca	
Cd^{2+} (pH 7–7.5)	–	16.05	–	–	–	55.5
$Cd^{2+}/(H_2PO_4^- + HPO_4^{2-})$	3:2	0.73	3.24	–	–	98.0
Cd^{2+}/HCO_3^-	1:1	0.15	–	–	–	99.6
Cd^{2+}/HS^-	1:1	1.29	–	–	–	97.0
$Cd^{2+}/(H_2PO_4^- + HPO_4^{2-})$ /Ca^{2+}	3:2:3	0.66	2.49	–	9.15	98.2
$Cd^{2+}/HCO_3^-/Ca^{2+}$	1:1:1	0.12	–	–	6.57	99.7
$Cd^{2+}/(H_2PO_4^- + HPO_4^{2-})$ /Fe^{3+}	3:2:2	0.48	1.06	≤0.01	–	98.7
$Cd^{2+}/HCO_3^-/Fe^{3+}$	3:3:2	0.08	–	≤0.01	–	99.8

[a] Initial concentration of Cd^{2+} = 36.05 mg/L, total volume of solution = 250 mL, pH 6–7 (except specified), temperature = 30°C, settlement time = 24 h
[b] Na^+ H^+, OH^- or NO_3^- balancing charges are not listed.

Table S12
Comparison of growth status of *Sedum Spectabile* with and without Cu^{2+} stress[a]

Cultural environment	Culture medium	Height of the plant before cultivation (cm)	Height of the plant after cultivation (cm)
With Cu^{2+} stress[b]	A	27	31
	B	29	31
	C	28	30
	D	27	28
Without Cu^{2+} stress	A	30	34
	B	29	33
	C	30	36
	D	30	36

[a] The duration of the cultivation is 9 weeks (without Cu^{2+} stress in the first week) , 25–35°C, and total volume of culture solution is 200 mL.
[b] Every 7 days, about 20 mg of Cu^{2+} ions was added into the culture medium, and totally 160 mg of Cu^{2+} ions was added.

Table S13
Comparison of Cu contents in the leaves of *Sedum Spectabile* cultivated under Cu^{2+} stress[a]

Elements	Contents of elements in *Sedum Spectabile*'s leaves cultivated under Cu^{2+} stress (mg/g) [b]				Contents of elements in *Sedum Spectabile*'s leaves cultivated without Cu^{2+} stress (mg/g)			
	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D
Cu	≤0.02	≤0.02	0.03	≤0.02	≤0.02	≤0.02	≤0.02	≤0.02

[a] The duration of the cultivation is 9 weeks, 25–35°C, and total volume of culture solution is 200 mL.
[b] Every one week, 20 mg of Cu^{2+} ions was added into the culture medium, and totally 160 mg of Cu^{2+} ions was added.

Table S14
Comparison of growth status of *Sedum Spectabile* with and without Ni^{2+} stress[a]

Cultural environment	Culture medium	Height of the plant before cultivation (cm)	Height of the plant after cultivation (cm)
With Ni^{2+} stress[b]	A	38	40
	B	35	40
	C	36	39
	D	37	39
Without Ni^{2+} stress	A	30	34
	B	29	33
	C	30	36
	D	30	36

[a] The duration of the cultivation is 9 weeks (without Ni^{2+} stress in the first week), 25–35°C, and total volume of culture solution is 200 mL.
[b] Every 7 days, 20 mg of Ni^{2+} was added into the culture medium, and totally 160 mg of Ni^{2+} was added.

Table S15
Comparison of Ni contents in the leaves of *Sedum Spectabile* cultivated under Ni^{2+} stress[a]

Elements	Contents of elements in *Sedum Spectabile*'s leaves cultivated under Ni^{2+} stress (mg/g)[b]				Contents of elements in *Sedum Spectabile*'s leaves cultivated without Ni^{2+} stress (mg/g)			
	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D
Ni	0.24	0.06	0.05	≤0.02	≤0.02	0.03	≤0.02	≤0.02

[a] The duration of the cultivation is 9 weeks, 25–35°C, and total volume of culture solution is 200 mL.
[b] Every one week, 20 mg of Ni^{2+} ions was added into the culture medium, and totally 160 mg of Ni^{2+} ions was added.

Table S16
Comparison of growth status of *Sedum Spectabile* with and without Mn^{2+} stress [a]

Cultural environment	Culture medium	Height of the plant before cultivation (cm)	Height of the plant after cultivation (cm)
With Mn^{2+} stress [b]	A	34	35
	B	34	37
	C	33	37
	D	32	32
Without Mn^{2+} stress	A	30	34
	B	29	33
	C	30	36
	D	30	36

[a] The duration of the cultivation is 9 weeks (without Mn^{2+} stress in the first week), 25–35°C, and total volume of culture solution is 200 mL.
[b] Every 7 days, 20 mg of Mn^{2+} was added into the culture medium, and totally 160 mg of Mn^{2+} was added.

Table S17
Comparison of Mn contents in the leaves of *Sedum Spectabile* cultivated under Mn^{2+} stress[a]

Elements	Contents of elements in *Sedum Spectabile*'s leaves cultivated under Mn^{2+} stress (mg/g)[b]				Contents of elements in *Sedum Spectabile*'s leaves cultivated without Mn^{2+} stress (mg/g)			
	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D
Mn	0.10	0.03	0.34	0.05	≤0.02	≤0.02	≤0.02	≤0.02

[a] The duration of the cultivation is 9 weeks, 25–35°C, and total volume of culture solution is 200 mL.
[b] Every one week, 20 mg of Mn^{2+} ions was added into the culture medium, and totally 160 mg of Mn^{2+} ions was added.

Table S18
Comparison of growth status of *Sedum Spectabile* with and without Zn^{2+} stress[a]

Cultural environment	Culture medium	Height of the plant before cultivation (cm)	Height of the plant after cultivation (cm)
With Zn^{2+} stress[b]	A	28	33
	B	29	30
	C	27	33
	D	27	28
Without Zn^{2+} stress	A	30	34
	B	29	33
	C	30	36
	D	30	36

[a] The duration of the cultivation is 9 weeks (without Zn^{2+} stress in the first week), 25–35°C, and total volume of culture solution is 200 mL.
[b] Every 7 days, about 20 mg of Zn^{2+} ions was added into the culture medium, and totally 160 mg of Zn^{2+} ions was added.

Table S19
Comparison of Zn contents in the leaves of *Sedum Spectabile* cultivated under Zn^{2+} stress[a]

Elements[b]	Contents of elements in *Sedum Spectabile's* leaves cultivated under Zn^{2+} stress (mg/g)[c]				Contents of elements in *Sedum Spectabile's* leaves cultivated without Zn^{2+} stress (mg/g)			
	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D	Cultural medium A	Cultural medium B	Cultural medium C	Cultural medium D
Zn	≤0.02	≤0.02	≤0.02	≤0.02	≤0.02	0.03	≤0.02	≤0.02

[a] The duration of the cultivation is 9 weeks, 15–25°C, and total volume of culture solution is 200 mL.
[b] Every one week, 20 mg of Zn^{2+} ions was added into the culture medium, and totally 160 mg of Zn^{2+} ions was added.

Table S20
Comparison of Cu^{2+} adsorption in the presence of Citrate[a]

Adsorbents[b]	Final concentration of Cu^{2+} in solution (mg/L)	Cu^{2+}-removed (%)
Calcium phosphate	2.04	59.2
Calcium carbonate	4.37	12.6
Iron(II) sulfide	2.38	52.4
Cysteine	3.49	30.2
Cystine	0.06	98.8
	0.42[c]	91.6[c]

[a] Initial concentration of Cu^{2+} in solution = 5.0 mg/L, molar ratio of citric acid to Cu = 5:1, amount of adsorbents = 0.1 g, total volume of solution = 50 mL, pH = 7–7.5, exchange time = 24 h, temperature = 20°C

[b] The grain-size was about 65–74 μm

[c] Molar ratio of citric acid to Cu = 10:1

Table S21
Comparison of some organic-acids commonly existing in plants in terms of dissolution of copper phosphate or nickel phosphate[a]

Organic-Acids	pH	Final concentration of Cu^{2+} in solution (mg/L)	Final concentration of Ni^{2+} in solution (mg/L)
Citric acid	3–3.5	565.5	401.8
	8–8.5	376.1	293.4
Tartaric acid	3–3.5	507.5	325.4
	8–8.5	276.8	147.5
Malic acid	3–3.5	505.8	369.4
	8–8.5	290.4	208.8

[a] Amount of copper phosphate (Cu^{2+} content in it = 34.6 wt %) or nickel phosphate (Ni^{2+} content in it = 22.9 wt %) is 0.1 g, molar ratio of an organic-acid to Cu^{2+} or Ni^{2+} is 5:1, total volume of solution is 60 mL, dissolution time is 24 h, temperature is ca. 25°C.