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Cadmium accumulation and apoplastic and symplastic transport in *Boehmeria nivea* (L.) Gaudich on cadmium-contaminated soil with the addition of EDTA or NTA

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A Cd-tolerant plant species named *Boehmeria nivea* (L.) Gaudich (ramie) was applied to study its Cd accumulation and translocation mechanisms with the addition of ethylene diamine tetracetic acid (EDTA) or nitrilotriacetic acid (NTA). A pot experiment was designed to systematically investigate the Cd accumulation and subcellular compartmentation in different ramie tissues as well as soil Cd solubility and its physiological response. Results showed that soil EDTA- and NTA-extractable Cd concentrations were remarkably higher than the control, and Cd content in each tissue with Cd translocation factor (TF) after EDTA and NTA addition were significantly increased with elevated chelant concentration. In spite of the decreased cytoderm Cd contents in different tissues, extracellular and intracellular Cd content were increased dramatically under chelant treatment, particularly in ramie leaves with EDTA addition from 2 mmol kg^{-1} to 10 mmol kg^{-1} (increased by 98% for extracellular Cd and by 29% for intracellular Cd, respectively). Furthermore, the addition of chelant also resulted in an apparent increase of malondialdehyde (MDA) content and decrease of chlorophyll level in ramie leaves. These results revealed that EDTA and NTA could enhance Cd phytoavailability in soil, facilitate apoplastic and symplastic transport of Cd from root to the aboveground tissues and improve leaf Cd accumulation, which may because of the extracellular loading among spongy tissues and intracellular sequestration in mesophyll vacuoles. This study contributes to the control of Cd accumulation by plants.

1. Introduction

Cadmium (Cd) is a major anthropogenic pollutant derived from agricultural and industrial practice, wastewater irrigation, and smelter waste and residues from the mining and smelting of metalliferous ores.[1] Due to its non-degradability, chemical mobility and high toxicity to biota, Cd can transfer through food chains and then cause various diseases to plants, animals and even human beings.[2] Given that Cd contamination has posed an unprecedented threat to a wide range of ecosystem and human health, more and more attention has been globally focused on the mechanisms of Cd contamination and remediation technologies.

Phytoextraction, the utilization of plants to transport and concentrate metals from soil into the harvestable parts of plants, is considered to be one of the most cost-effective and environmental-friendly strategies to remediate heavy metal contamination.[3] Hyperaccumulators are proposed to be applied in phytoextraction technology since they are naturally capable of not only surviving in soil with high heavy metal concentrations, but also accumulating heavy metals to an exceedingly high level in their aboveground tissues.[4] Nevertheless, the strategy with hyperaccumulator usually fails to achieve the expected remediation effect due to their low biomasses, low growth rates and strict growing conditions.

Phytoextraction with non-hyperaccumulators is described as three subsequent levels: transfer of metals from the bulk soil to the root surfaces, absorption into the roots and translocation to the shoots.[5] In the rhizosphere, soil metals bioavailability tend to be increased by roots-excreted metal-chelating molecules or protons that can acidify the soils and hence enhance metal availability to plant roots.[6] Transport across root cellular membrane is another important process that initiates metal absorption into plant tissues. The concentration dependence of Cd absorbed by either excised roots or intact plants from hydroponic solutions generally follows the sum of a linear component and a single Michaelis–Menten component.[7,8] The

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linear component represents tight Cd binding to cell walls and an extracellular Cd,[9] while the Michaelis–Menten component suggests an intracellular Cd absorbed *via* a carrier-mediated system.[10] That is to say, Cd may traverse the root to the shoot either through the extracellular spaces between cells (apoplast) or through the cytoplasmic continuum of root cells linked by plasmodesmata (symplast).[9] However, exposure to excess Cd has been found to accelerate root maturation and result in the formation of Casparian bands and suberin lamellae closer to the root apex, which forms the physical barriers to the apoplastic movement of Cd in root and to shoot.[11,12] Symplastic transport of Cd is also limited by Cd sequestration in root vacuoles.[13] Hence, phytoextraction with non-hyperaccumulators achieves a low remediation efficiency in removing heavy metals from contaminated soil for the reason that non-hyperaccumulators mainly accumulate heavy metals in the roots rather than in the aboveground tissues.

Chelant-enhanced phytoextraction has revolutionized phytoextraction technology by utilizing chelating agents to improve the remediation efficiency of non-hyperaccumulator with large biomass, deep root and high growing rate.[14] On one hand, chelant may facilitate the dissolution of soil Cd and make it available to be absorbed by plant roots. On the other hand, the addition of chelant may break the physical barriers in plant roots and adjust the transport pathway of Cd in plant, thereby enhancing the translocation of Cd in plant tissues.

Of the chelants investigated, ethylene diamine tetracetic acid (EDTA) is proved to be the most effective chelating agent, which is widely applied to remediate heavy metal contaminated soil.[15–18] However, due to the low biodegradability and high solubility, EDTA may result in high environmental risk of heavy metal leaching to groundwater.[19] Recently, the focus of researches on chelant-enhanced phytoextraction has been shifted to some biodegradable chelants such as nitrilotriacetic acid (NTA), for its use in improving the uptake of metals by plants and in limiting metal leaching into deeper soils.[20] Several studies have been performed using NTA as a ligand to improve metal phytoextraction. As reported early, NTA performed effectively in desorbing Cu, Pb and Zn from soils,[21] increasing Cu, Pb and Zn uptakes in shoots of *Festuca arundinacea*[19] and improving Cd accumulation and translocation in *Siegesbeckia orientalis*.[22]

The technology of phytoextraction has been into practical for several years and the market for phytoextraction of metals from soils in the USA alone was approximately $70–100 million by 2005.[23] Regarding the chelant-enhanced phytoextraction, although EDTA is an interesting model for enhanced phytoextraction research, EDTA has probably a low applicability in practical field application due to unacceptable leaching risks associated with its environmental persistence.[24] To the level of practical field scale application, the shift is made towards more degradable alternatives incorporating the principle of improving metal phytoavailability for phytoextraction purposes.

Boehmeria nivea (L.) Gaudich (ramie), namely "Chinese grass", is one of the best plant fibers for textile and mainly distributes in tropical and semitropical area from the south to Qinling Mountain of China.[25] The previous studies have identified ramie as a Cd-tolerant species with large biomass and fast growth rate, and then its tolerance mechanism towards Cd has been well investigated.[26,27] However, little information is available about the accumulation and translocation mechanisms of Cd in ramie in the presence of chelant. Therefore, in this study, we investigated the effects of non-biodegradable EDTA and biodegradable NTA on soil Cd solubility, Cd phytotoxicity in ramie, and Cd accumulation in different ramie tissues. To further study the accumulation and translocation mechanisms of Cd at subcellular level, we also determined the distribution of Cd in the extra- and intra-cellular compartments and the cytoderm of ramie roots, stems and leaves after the addition of EDTA or NTA.

2. Materials and methods

2.1. Soil preparation

The experimental soil was collected from the superficial layer (0–20 cm), originated from Yuelu Mountain, which located at Hunan University (Changsha, China) research area. The fresh soil was air-dried, homogenized and sieved through a 2 mm screen sieve. In all treatments, the soil was amended at 50 mg kg^{-1} Cd with $Cd(NO_3)_2 \cdot 4H_2O$ and then incubated for four weeks. In incubation, the relative humidity of soil was adjusted with deionized water (DIW) to 60% of water holding capacity (WHC). Basal fertilizers were applied as KH_2PO_4 and NH_4NO_3 to the soil with 80 mg kg^{-1} P, 100 mg kg^{-1} K and 100 mg kg^{-1} N.

2.2. Extraction of Cd from soil by EDTA or NTA

The ability of chelants to extract Cd from the contaminated soil was examined using a consecutive solubilization approach as suggested by Quartacci *et al.*[21] with some modifications. Chelate-extracting solutions of EDTA or NTA were prepared in different concentrations of 0, 1, 5, 10, 20 and 30 mM. 0 represented the control group without treatment of EDTA or NTA. For each treatment, 5.0 g soil was placed in a 50 mL polypropylene centrifuge tube and then 30 mL extracting solution was added. Samples were capped, shaken for 16 h, and then centrifuged at 3000*g* for 10 min. The supernatants were collected and analyzed for Cd concentration flame atomic absorption spectrometry (FAAS, Perkin-Elmer, AA700, USA).

2.3. Plant materials and growth conditions

2.0 kg of Cd-amended soil was filled into 3.0 L plastic pots. One-month ramie seedlings with comparable height and biomass, collected from Institute of Bast Fiber Crops (Hunan Agricultural University, Changsha, China), were grown in pot (three seedlings for each pot) and acclimated for two weeks. Afterwards, the pots were correspondingly treated with different concentrations of EDTA or NTA (0, 2, 5, and 10 mmol kg^{-1} soil in a 200 mL solution) to the surface of the soil. 0 represented the control group with treatment of 50 mg kg^{-1} Cd alone. Ramies treated without Cd and chelant were also prepared for making a comparison in ramie physiological experiment. All experiments were conducted in a controlled greenhouse with the following conditions: 16 h day length with a photon flux density of

350 μmol m^{-2} s^{-1}, day/night temperature of 25 °C/20 °C and 60–70% humidity. After two weeks, the plants were harvested and separated into roots, stems and leaves, and frozen in −80 °C for further analysis.

2.4. Determination of Cd in ramie tissues

Ramie tissues were dried at 80 °C and digested in a mixture of concentrated HNO_3 and $HClO_4$ (4 : 1, by volume) by graphite digestion instrument (SISP, DS-360, China). The Cd concentration of each solution was determined by FAAS. Translocation factor (TF) is defined as the total metal content in plant stem or leaf to that in the root.

2.5. Isolation of the extracellular, intracellular and cytoderm Cd of ramie cells

The Cd distribution in extracellular, intracellular and cytoderm were measured to distinguish the selective symplastic transport and nonselective apoplastic transport influenced by different chelants. After harvest, the roots, stems and leaves of ramie were separated and then desorbed using a modified desorption procedure as described in Zhang *et al.*[28] The plant tissues from different chelant treatments were rinsed using 5.0 mM $CaCl_2$ and DIW to remove the metals adsorbed on the surface. Then they were desorbed in 5.0 mM $CaCl_2$, which was refreshed every five min. After 20 min, most of the extracellular metals were removed, the samples were rapidly frozen in liquid nitrogen to disrupt cell membranes and the desorption was continued for 40 min. The metals released in the first 20 min desorption and in the continued 40 min desorption following the freeze–thaw process were considered as extracellular fraction and intracellular fraction, respectively. The metals remaining in the root (mainly binding or precipitated in the root cell wall) after the freeze–thaw was considered as cytoderm fraction.

2.6. Determination of chlorophyll and malondialdehyde (MDA) content

The chlorophyll content of ramie leaf was determined using the acetone method.[29] Frozen leaf tissues were homogenized in 80% ice-cold acetone in dark and then centrifuged at 2000*g* for 10 min. Then, chlorophyll content was determined spectrophotometrically on the supernatant at wavelength of 646 nm and 663 nm.

The MDA content of leaves was determined using the thiobarbituric acid (TBA) method.[30] Plant leaf (0.5 g) was homogenized with 10 mL 10% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000*g* for 10 min. Then 2.0 mL of the aliquot of the supernatant and 2.0 mL of 10% TCA containing 0.5% (w/v) TBA were added. The mixture was incubated at 95 °C for 30 min and then cooled quickly in an ice-bath. The samples were centrifuged at 10 000*g* for 15 min and the absorbance of the supernatant was measured at 532 nm and corrected for nonspecific absorbance at 600 nm. The concentration of MDA was calculated using 155 mM^{-1} cm^{-1} as extinction coefficient.

2.7. Statistical analysis

The data were subjected to SPSS 20.0 for statistical analyses, and then one-way analysis of variance (ANOVA) followed by Tukey HSD ($P < 0.05$) was preformed. The results from a representative experiment were presented as mean values ± SD of four replications.

3. Results

3.1. Soil Cd extraction analysis under EDTA or NTA treatment

The ability of EDTA or NTA to extract Cd from Cd-contaminated soil was illustrated in Fig. 1. Cd concentrations extracted by EDTA and NTA extractants were significantly increased ($P < 0.05$) as a function of chelant concentration, remarkably ($P < 0.05$) exceeding that in the control. However, the two chelants varied greatly in the capacity to extract Cd from the soil and the Cd increase became more pronounced ($P < 0.05$) in EDTA group. Compared to NTA, the addition of EDTA exhibited a higher rate of Cd extraction at all treatment levels, approximately 1.12- to 2.73-fold the Cd concentration extracted by NTA. The results indicated that both two kinds of chelants were capable of extracting significant amounts of Cd from soil and EDTA was more effective in activating soil Cd compared to NTA.

3.2. Effect of EDTA and NTA on Cd accumulation and translocation in ramie

The Cd concentrations in different tissues of ramie cultivated in Cd-amended soil after the addition of EDTA or NTA as well as the TF values were summarized in Table 1. In EDTA group, there were visible increases ($P < 0.05$) in Cd contents of different tissues compared to the control, and a marked increase in leaf Cd was recorded, nearly 5.21- to 6.74-fold higher than the control. Cd contents in different tissues of ramie shared an increasing trend with EDTA supply and ranked as follows: root > stem > leaf for the control group and leaf > root > stem for the EDTA group, respectively. In NTA group, Cd contents in ramie

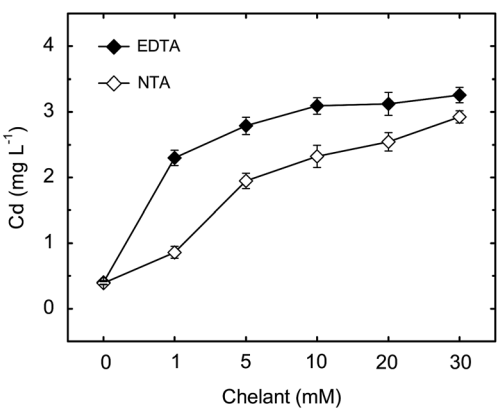

Fig. 1 The Cd concentration of extracted solution from Cd-contaminated soil by various concentrations of EDTA or NTA (0–30 mM). 0 represents the control group with treatment of 50 mg kg^{-1} Cd alone. Data represent means ± SD of four replicates.

Table 1 The Cd content in different ramie tissues and the TF value after the addition of various concentrations of EDTA or NTA (0–10 mmol kg^{-1})[a]

Chelant addition (mmol kg^{-1})	Cd content (mg kg^{-1} DW)			TF value	
	Root	Stem	Leaf	Stem	Leaf
0	165.54 ± 8.563	73.87 ± 3.642	37.42 ± 1.556	0.44 ± 0.010	0.23 ± 0.001
2 mmol kg^{-1} EDTA	182.66 ± 8.989	94.97 ± 4.364	233.60 ± 11.294	0.52 ± 0.003	1.28 ± 0.011
5 mmol kg^{-1} EDTA	196.83 ± 9.403	116.71 ± 4.969	288.60 ± 12.001	0.59 ± 0.001	1.47 ± 0.009
10 mmol kg^{-1} EDTA	201.88 ± 8.305	119.74 ± 4.494	270.16 ± 10.452	0.59 ± 0.001	1.34 ± 0.012
2 mmol kg^{-1} NTA	115.63 ± 4.016	67.18 ± 2.964	94.56 ± 5.204	0.58 ± 0.006	0.82 ± 0.009
5 mmol kg^{-1} NTA	100.23 ± 5.370	95.75 ± 4.618	90.43 ± 4.218	0.95 ± 0.011	0.90 ± 0.013
10 mmol kg^{-1} NTA	120.30 ± 5.852	106.39 ± 4.666	85.87 ± 4.313	0.88 ± 0.001	0.71 ± 0.008

[a] 0 represents the control group with treatment of 50 mg kg^{-1} Cd alone. Data represent means ± SD of four replicates.

stems and leaves were significantly ($P < 0.05$) higher than that in the control group while Cd contents in ramie roots were conversely lower than the control. There was no obvious fluctuation ($P > 0.05$) in Cd content of each ramie tissue between various NTA concentrations and the highest Cd concentration occurred in roots, followed by leaves or stems. Furthermore, the higher Cd contents existed in ramie tissues treated with EDTA as compared to NTA. In particular, Cd content in ramie leaves was increased from 233.60 to 288.60 mg kg^{-1} DW as a function of the EDTA concentration, 1.47- to 2.19-fold higher than that in the NTA group.

In general, it is accepted that plant species are classified as excluders with TF < 1, while as accumulators and hyperaccumulators with TF > 1.[31,32] According to Table 1, the mean TF value of stem gradually varied from 0.52 to 0.59 in EDTA group and from 0.58 to 0.95 in NTA group, significantly ($P < 0.05$) exceeding that in the control (0.44). Compared to the NTA group, TF of leaf with values exceeding 1 in EDTA group obtained a more dramatical rise ($P < 0.05$), nearly close to the level of hyperaccumulators. Ultimately, EDTA was more available than NTA to stimulate the transport efficiency of Cd from root to the aboveground tissues.

3.3. Effect of EDTA and NTA on Cd subcellular compartmentation in ramie

Cd concentrations in extra- and intra-cellular compartments and cytoderm in separated ramie tissues under Cd stress with EDTA or NTA addition were determined to evaluate the effect of chelant on Cd subcellular distribution in ramie cells (Fig. 2–4). The Cd subcellular distributions in different plant parts varied approximately in the order of root > stem > leaf in the control group and leaf > root > stem in the chelant groups, respectively. In EDTA group, the extracellular and intracellular Cd contents in each tissue were observed to increase gradually ($P < 0.05$) with increasing EDTA concentration. Especially, extracellular and intracellular Cd contents in ramie leaves were increased from 62.70 to 179.54 mg kg^{-1} FW and from 169.42 to 218.52 mg kg^{-1} FW respectively, while Cd was undetectable in extracellular and intracellular fraction of ramie leaves from the control (Fig. 4). Besides, cytoderm Cd contents in ramie roots and stems were

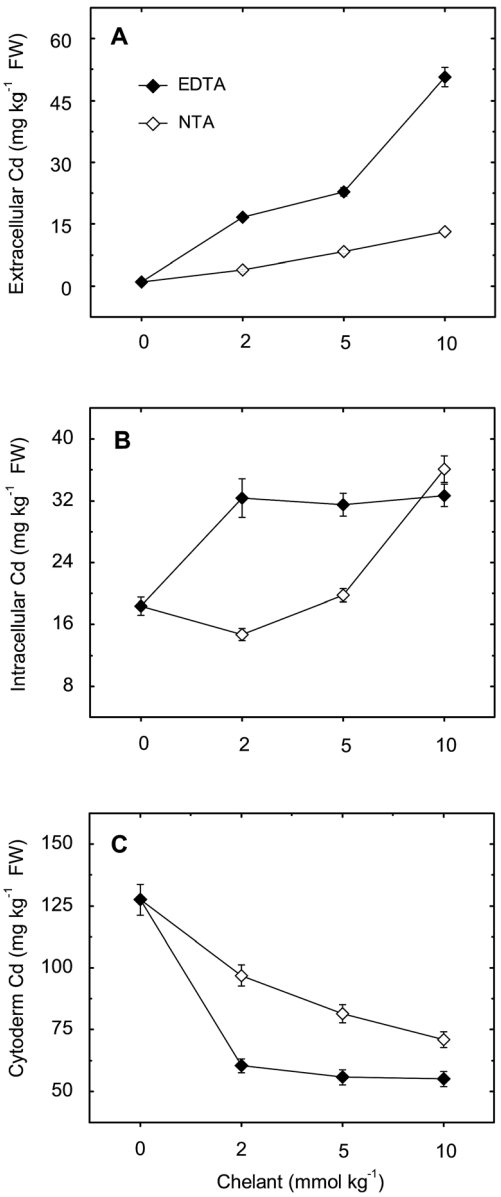

Fig. 2 The extracellular Cd (A), intracellular Cd (B) and cytoderm Cd (C) of ramie roots after the addition of various concentrations of EDTA or NTA (0–10 mmol kg^{-1}). 0 represents the control group with treatment of 50 mg kg^{-1} Cd alone. Data represent means ± SD of four replicates.

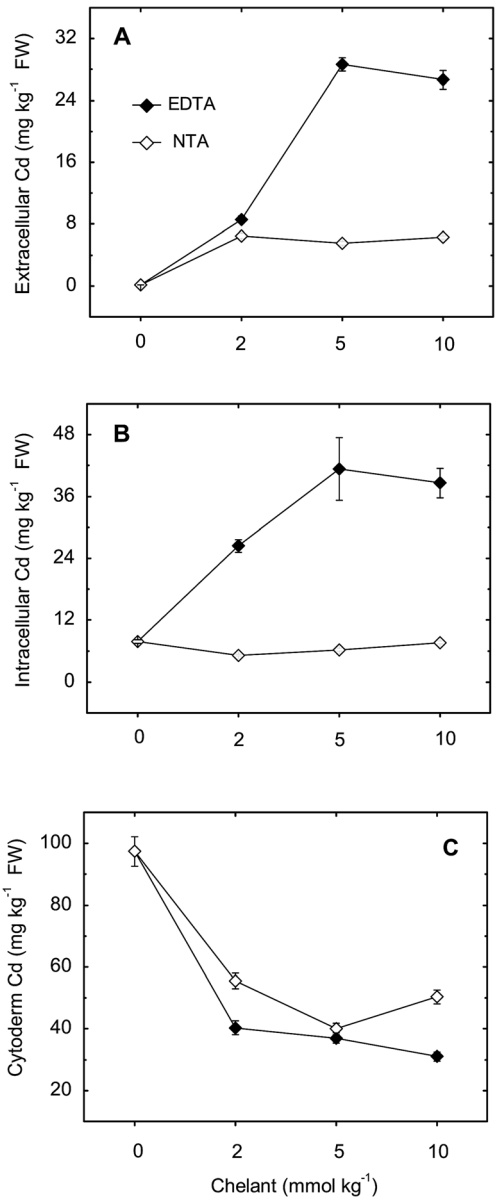

Fig. 3 The extracellular Cd (A), intracellular Cd (B) and cytoderm Cd (C) of ramie stems after the addition of various concentrations of EDTA or NTA (0–10 mmol kg^{-1}). 0 represents the control group with treatment of 50 mg kg^{-1} Cd alone. Data represent means ± SD of four replicates.

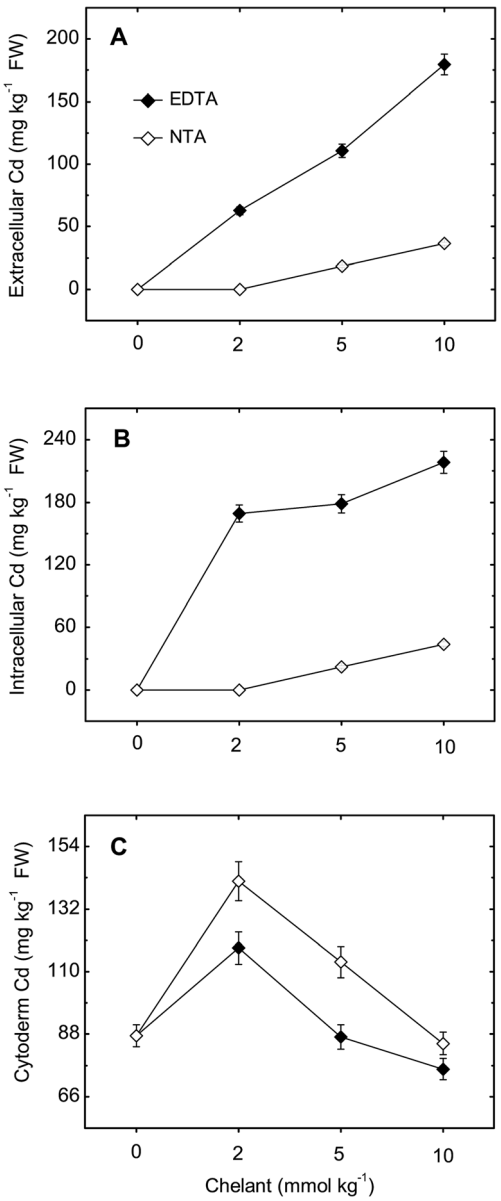

Fig. 4 The extracellular Cd (A), intracellular Cd (B) and cytoderm Cd (C) of ramie leaves after the addition of various concentrations of EDTA or NTA (0–10 mmol kg^{-1}). 0 represents the control group with treatment of 50 mg kg^{-1} Cd alone. Data represent means ± SD of four replicates.

decreased significantly ($P < 0.05$) as the EDTA concentration increased (Fig. 2C and 3C). However, in ramie leaves, the cytoderm Cd content at low EDTA concentration (2 mmol kg^{-1}) was 35% higher ($P < 0.05$) than that in the control (87.44 mg kg^{-1} FW), but an obvious decrease was observed (from 118.34 to 75.68 mg kg^{-1} FW, $P < 0.05$) with further rising EDTA concentration. In NTA group, extracellular and intracellular Cd contents in separated ramie tissues were remarkably ($P < 0.05$) lower than those in EDTA group, although they kept an uptrend with rising NTA concentration. Particularly, in ramie leaves, the highest extracellular and intracellular Cd content at 10 mmol kg^{-1} NTA were only 21% and 20% of that at 10 mmol kg^{-1} EDTA, respectively. Moreover, the cytoderm Cd content in NTA group presented a similar trend to that in EDTA group, and there was no obvious ($P > 0.05$) distinction between the two kinds of chelant in terms of cytoderm Cd.

3.4. Effect of EDTA and NTA on ramie physiology

All the ramie seedings in EDTA group exhibited phytotoxicity symptoms after two weeks of chelant treatment with chlorosis and black spot present in leaves, whereas only high dose of NTA caused a negative modification on physiological trait in ramie leaves. The toxic effects of EDTA and NTA on ramie were also reflected by the alteration of the chlorophyll content and the MDA content in ramie leaves.

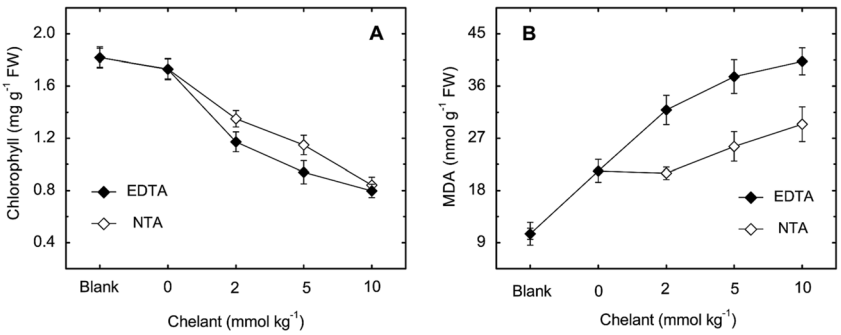

Fig. 5 Total leaf chlorophyll content (A) and MDA content (B) in ramie leaves treated with various concentrations of EDTA or NTA (0–10 mmol kg^{-1}). Blank represents the ramie group cultivated in soil without Cd and chelant treatment. 0 represents the control group with treatment of 50 mg kg^{-1} Cd alone. Data represent means ± SD of four replicates.

The leaf chlorophyll was determined to elucidate the toxic effect of Cd or exogenous chelants on ramie photosynthesis system (Fig. 5A). Chlorophyll content in ramie exposed to 50 mg kg^{-1} Cd (the control) showed no significant alteration ($P > 0.05$) from that in ramie treated without Cd and chelant, but was greatly ($P < 0.05$) higher than that in ramie treated with EDTA or NTA. Furthermore, chlorophyll content decreased by 54% in response to EDTA addition from 0 to 10 mmol kg^{-1}, which has a similar depletion rate in NTA group (by 51%) within the same chelant concentration range.

The concentration-responses of MDA content to EDTA or NTA were given in Fig. 5B, which reflected the degree of cell membrane damage caused by oxygen free radicals.[31] MDA content was 10.5 nmol g^{-1} FW in ramie treated without Cd and chelant but reached up to 21.4 nmol g^{-1} FW when ramies were exposed to Cd of 50 mg kg^{-1} (control). MDA content in EDTA group ranged from 31.9 to 40.3 nmol g^{-1} FW, significantly higher ($P < 0.05$) than that in the control group. Nevertheless, compared to the control, there was no obvious increase ($P > 0.05$) in MDA content at low NTA concentrations (2 mmol kg^{-1}) but apparently a higher MDA content (29.4 nmol g^{-1} FW) was detected in ramie treated with 5 and 10 mmol kg^{-1} NTA.

4. Discussion

Cd concentration in plant root initially depends on the bioavailable Cd concentration in soil. As a result of the strong association of Cd with organic matters or Fe–Mn oxides, Cd appears to be mainly bound to the residual fraction in soil,[34] which is difficult to dissolve in soil solution and can hardly be taken up by plant root. The addition of chelant greatly increased soil Cd dissolution (Fig. 1), thereby enhancing the Cd bioavailability to plant. Because of the strong chemical affinity for Cd, chelant can be employed to promote the remobilization of insoluble Cd from soil metal oxides and organic matter,[35] thus chelating with Cd in soil solution and forming stable Cd–chelate complex. EDTA has a relatively higher chemical affinity for Cd ($\log K_s = 16.44$) compared with NTA ($\log K_s = 9.80$),[36] resulting in the higher efficiency in increasing the Cd concentration in soil extraction (Fig. 1). Besides, the experimental soils in this research were mainly the unnatural soil artificially enhanced with Cd, which had a larger mobility and bioavailability of Cd compared to the naturally contaminated soils.

Miller *et al.*[37] suggested that a portion of metal cations that penetrated roots were bound in plant cell walls and the rest accumulated in the extracellular space, due to the cation exchangeable sites on the cell wall and extracellular deposition mainly in the form of metal carbonates. In addition, the metal cations escaping from binding to cell wall could pass through the cell membrane *via* the ZRT/IRT-like protein (ZIP) transporters or cation channels,[38] traverse the tonoplast through Cd/H antiporters and then accumulate in intracellular space by chelating metal ions with glutathiones, metallothioneins and phytochelatins induced by metal cations.[39] As a result, in the presence of high Cd concentration, the upward movement of Cd cations to aboveground tissues is limited although plant has accumulated considerable Cd in roots.

The addition of chelant was shown to increase the Cd uptake by ramie roots and even promote the Cd accumulations in ramie stems and leaves (Table 1), which is in good agreement with Meighan *et al.*[17] and Lan *et al.*[22] In the presence of chelant, soil metals are mainly in the form of metal–chelate complexes, which can readily enter the plant root by destroying the Casparian strip in root endodermis.[5,40,41] According to Fig. 2C, in the presence of chelant, Cd accumulation suffered a sharp decrease in cytoderm of ramie roots, due to the reason that the absorbed Cd in the form of metal–chelate complexes has little affinity for the cation exchangeable sites on the cell wall. This can also account for the cytoderm Cd depletion in ramie stems and leaves under chelant treatment (Fig. 3C and 4C). In addition, the extracellular and intracellular Cd in root cells achieved a rise after the addition of chelant (Fig. 2A and B). Free of the trammel of cell walls, a great portion of Cd–chelate complexes in ramie roots could, more readily and freely, transport in extracellular space by apoplastic pathway or traverse the intracellular space by symplastic pathway.[42] On the other hand, another portion of Cd–chelate complexes could directly enter into the cytoplasm through Yellow-Stripe 1-Like (YSL) proteins or accumulate in the root cell vacuoles by passing through the tonoplast *via* ATP-binding cassette (ABC) transporters.[43]

Following Cd uptake by roots, xylem loading of Cd is regarded as the next important transport process of Cd accumulation in plant. Metals could reach the xylem through either the symplastic or the apoplastic bypass. Symplastic bypass is considered as an energy-consuming pathway involving metal transporter protein, while apoplastic bypass is known to be a passive pathway correlated with transpiration.[44] In this study, the extracellular and intracellular Cd content in ramie stems significantly increased with increasing chelant concentration, but much less than those in ramie leaves (Fig. 3 and 4). The reason may be assumed that Cd–chelates transported to the ramie leaves by both pathways were improved but with little accumulation in stems.

The extracellular and intracellular Cd in ramie leaves obtained a remarkable increase after the addition of chelant (Fig. 4A and B). On one hand, the loosened spongy tissues (a dominating part in plant leaves) have a large extracellular space to gather Cd or Cd–chelate complexes. On the other hand, abundant vacuoles in mesophyll cells can not only provide a huge space for storing excess Cd or Cd–chelate complexes, but also filter and sequester potentially toxic Cd cations from the cytosol, making them unavailable for interaction with metabolically active cellular compartments.[45]

There existed a big discrepancy on Cd accumulation and translocation between EDTA group and NTA group. Total Cd contents in all the ramie tissues as well as TF values in stem and leaf were apparently lower in NTA group than that in EDTA group (Table 1), indicating that NTA is less effective in facilitating Cd accumulation and translocation in ramie. Additionally, in the NTA group, the extracellular and intracellular Cd in all the ramie tissues tended to be lower, but the cytoderm Cd appeared to be higher compared to those in the EDTA group (Fig. 2–4). Given that the affinity of NTA for Cd is of middle level, Cd–NTA chelates are not as stable as Cd–EDTA chelates. It is suggested that Cd could be desorbed from Cd–NTA chelates in ramie cells, and then resorbed by the cytoderm substance with higher affinity. Furthermore, considering the biodegradability of NTA, free NTA and NTA chelated with Cd tend to be biodegraded after the function of related enzymes.[46] Thus, free Cd cations were again bound to cell walls. This could also explain that the cytoderm Cd contents were significantly higher than the extracellular and intracellular Cd contents in the presence of NTA (Fig. 2C, 3C and 4C).

Although chelants can have a positive effect on Cd accumulation and translocation in ramie, they also can exert side-effect on ramie physiology. It was reported that Cd interfered chlorophyll formation by interacting with functional –SH groups of sulfhydryl-requiring enzymes or increasing the activity of lipoxygenase,[47] which could cause oxidative damage. The lipoxygenase can also mediate the Cd-induced lipid peroxidation by causing the MDA accumulation when plants are subjected to oxidative stresses.[33] In present research, the addition of chelant resulted in an increase of MDA content (Fig. 5B) with concurrent decrease of chlorophyll (Fig. 5A) in ramie leaves, which is partially attributed to that the accumulation of chelant and Cd–chelate complexes in ramie leaves plays an important role in disturbing the lipoxygenase pathway and photosynthetic process.[40,48] Fig. 5 clearly shows that the symptoms of chlorophyll loss and lipid peroxidation in ramie leaves were enhanced with the concentrations of the chelants, which may be because of that NTA or EDTA alone has a side effect on plant physiology. However, because of the properties of hypotoxicity and biodegradability, NTA has a less phytotoxic effect on ramie physiology than EDTA, as was evidenced by the higher chlorophyll content (Fig. 5A) and lower MDA content in the NTA group (Fig. 5B). Although NTA is less effective than EDTA in facilitating Cd accumulation and translocation in ramie, its lower phytotoxicity suggests that NTA has a potential to be applied in chelant-enhanced phytoextraction.

In addition, to further probe the effect of chelants on Cd toxicity to plant physiology and Cd absorption by plant in natural soil environment, it is necessary to make a laboratory to field extrapolation in the subsequent research.

5. Conclusions

Both EDTA and NTA have positive effects on Cd accumulation and translocation in ramie. EDTA and NTA could enhance the bioavailability of soil Cd by chelating with them to form Cd–chelate complexes, and then improve the capability of ramie to take up and translocate Cd or Cd–chelate complexes from soil to the aboveground tissues despite of some phytotoxicity effect posed by chelants. Cd distributions among extra- and intracellular compartments and cytoderm in different ramie tissues further clarified that chelants could conduce to the accumulation and transport of Cd to some extent by enhancing the Cd symplastic and apoplastic transport in ramie tissues and promoting the Cd accumulation in extracellular space of leaf spongy tissues and in mesophyll vacuoles. Because of its lower affinity for Cd, NTA is less effective than EDTA in facilitating Cd accumulation and translocation in ramie. However, due to its properties of hypotoxicity and biodegradability, NTA is less toxic to ramie physiology. Therefore, NTA has a potential to replace EDTA in the application of chelant-enhanced phytoextraction.

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