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Stabilized Nanoscale Zerovalent Iron Mediated Cadmium Accumulation and Oxidative Damage of *Boehmeria nivea* (L.) Gaudich Cultivated in Cadmium Contaminated Sediments

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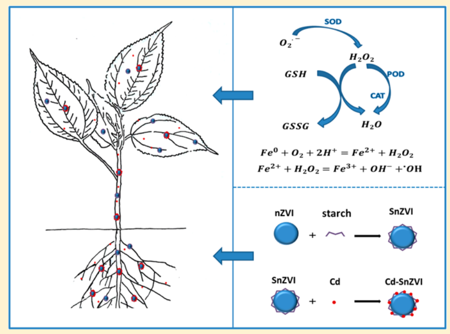
Supporting Information

ABSTRACT: Nanoparticles can be absorbed by plants, but their impacts on phytoremediation are not yet well understood. This study was carried out to determine the impacts of starch stabilized nanoscale zerovalent iron (S-nZVI) on the cadmium (Cd) accumulation and the oxidative stress in *Boehmeria nivea* (L.) Gaudich (ramie). Plants were cultivated in Cd-contaminated sediments amended with S-nZVI at 100, 500, and 1000 mg/kg, respectively. Results showed that S-nZVI promoted Cd accumulation in ramie seedlings. The subcellular distribution result showed that Cd content in cell wall of plants reduced, and its concentration in cell organelle and soluble fractions increased at S-nZVI treatments, indicating the promotion of Cd entering plant cells by S-nZVI. In addition, the 100 mg/kg S-nZVI alleviated the oxidative damage to ramie under Cd-stress, while 500 and 1000 mg/kg S-nZVI inhibited plant growth and aggravated the oxidative damage to plants. These findings demonstrate that nanoparticles at low concentration can improve the efficiency of phytoremediation. This study herein develops a promising novel technique by the combined use of nanotechnology and phytoremediation in the remediation of heavy metal contaminated sites.



■ INTRODUCTION

River sediments contaminated with heavy metals have become a widespread environmental concern because it is toxic to aquatic organisms and likely to cause water pollution.[1−3] Cadmium (Cd) contaminated sediments is becoming increasingly common due to the wide application of Cd in electroplating, metallurgy, and agriculture. Cd is a highly toxic heavy metal and can be bioaccumulated through food chain, which poses potential risks to the natural ecosystem and human health.[4−6] Consequently, remediation of Cd-contaminated sediments is imperative. Phytoremediation has been considered to be a cost-efficient technology for sediments remediation since it can remove the toxic substances from sediments, especially in the dry seasons of river.[7,8]

Nanomaterials are extensively used, which will inevitably have potential impacts on plants. For example, carbon nanotubes increased seeds germination rate and promoted the seedlings growth of rice plants;[9] plant photosynthetic activity was promoted by single-wall carbon nanotubes.[10] Some researchers reported the positive effects as mentioned above, whereas others found the negative effects of nanomaterials on plants. A study conducted by Lin and Xing[11] found that root elongation of corn was reduced by 35% at alumina nanomaterials treatments. Fullerene nanoparticles at high concentrations impeded plant development by interfering the uptake of water and nutrients.[12] The potential different effects of nanomaterials on plants certainly will affect the phytoremediation of the contaminated environment.

Nanomaterials have been applied to decontaminate pollutants in the past few years.[13−18] Recently, the combination of nanotechnology and phytoremediation to deal with different kinds of soil pollution has gained increasing concerns.[19] A previous study has shown that graphene oxide nanoparticles can serve as Cd carriers to promote the uptake of Cd in *Microcystis aeruginosa*.[20] *Panicum maximum* growing in the soil amended with 100, 500, and 1000 mg/kg nanoscale zerovalent iron (nZVI) resulted in higher trinitrotoluene accumulation.[21] nZVI at 750 and 1000 mg/kg promoted polychlorinated biphenyls accumulation and facilitated plant growth of *Impatiens balsamina*, thus enhancing the removal efficiency of soil contaminants.[22]

nZVI is a commonly used nanomaterial which has been widely applied for the remediation of metal contaminated soils and sediments. nZVI has high capacities for the removal/stabilization/degradation of environmental pollutants and can

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be separated by an external magnetic field.[23,24] For example, nZVI immobilized Cd in river sediments by transforming the mobile fraction of Cd into residual forms.[25] The interactions between nZVI and metals probably will affect the bioavailability of metals in plants. For example, nZVI at the dose of 1% and 10% were demonstrated to reduce the availability of arsenic (As) in soil and decrease the uptake of As in *Hordeum vulgare* L. cv. Pedrezuela;[26] stabilized nZVI enhanced Cr(VI) immobilization and decreased its bioaccumulation in Chinese cabbage.[27] In addition, previous reports[28,29] have confirmed the uptake of nZVI in plants. Since Cd can be adsorbed on iron hydroxides through physical sorption and surface complexation,[30] it is unclear whether nZVI can act as metal carriers to promote Cd accumulation and translocation in plants or it will immobilize Cd and reduce its bioavailability to plants.

Reactive oxygen species (ROS) are generated in plants as byproducts of aerobic metabolism (e.g., plant respiration and photosynthesis), which mainly include hydrogen peroxide (H_2O_2), hydroxyl radicals (OH●) and superoxide radicals (O_2●$^-$). The generation and scavenging of ROS in plants are balanced under general environmental conditions. Heavy metals, such as Cd, will produce excess ROS in plants, which may cause oxidative damage to proteins, DNA, and lipids. Previous studies have shown that nZVI can produce ROS through iron oxidation or Fenton reaction according to the following reactions:[31,32]

$$Fe^0 + O_2 + 2H^+ = H_2O_2 + Fe^{2+} \quad (1)$$

$$Fe^{2+} + H_2O_2 = OH\cdot + Fe^{3+} + OH^- \quad (2)$$

$$Fe^{2+} + O_2 = O_2^{\cdot -} + Fe^{3+} \quad (3)$$

In the presence of dissolved oxygen, iron is oxidized with the production of H_2O_2 (reaction 1). Furthermore, H_2O_2 converts to OH● through Fenton reaction (reaction 2). The generation of O_2●$^-$ can be achieved through ferrous ion oxidation (reaction 3). The nZVI-induced ROS generation or conversion may modify the oxidative stress in plants. However, it is unclear whether nZVI will aggravate Cd induced oxidative stress in plants. Moreover, the overproduction of ROS can be scavenged by antioxidative enzymes and some antioxidants. Previous investigations revealed that nanoparticles could modify the activities of enzymes related to the removal of ROS. For instance, cerium oxide nanoparticles were found to increase catalase (CAT) activity and reduce ascorbate peroxidase activity in cucumber.[33] nZVI changed the activities of superoxide dismutase (SOD) and lactate dehydrogenase (LDH) in *Escherichia coli* cells.[34] Furthermore, a study conducted by Chaithawiwat et al. even showed that nZVI particles performed roles in the expression of genes encoding antioxidative enzymes.[35] Such nanoparticles induced modification may change the antioxidative responses of plants exposed to heavy metals. To our knowledge, very few studies have investigated the impacts of nZVI on the antioxidant defense systems in plants under Cd-stress.

The current study focuses on the impacts of nZVI on the uptake and translocation of Cd in plants. In addition, the oxidative damage and the antioxidative defense of plants exposed to nZVI under Cd stress were investigated. Because of the highly reactivity, nZVI tended to either be oxidized or agglomerate. Previous studies have shown that starch can serve as stabilizer and dispersant to prevent nZVI from agglomeration and oxidation.[36] Thus, starch stabilized nZVI (S-nZVI) was used as the nanomaterial in the present experiments. In addition, our previous study revealed that *Bechmeria nivea* (L.) Gaud. (Ramie) is a promising species for the phytoremediation of Cd-contaminated environment due to its high Cd accumulation ability and large biomass.[37] Therefore, ramie seedlings were chosen as the test plants.

In the present study, plant dry weight (DW), metal concentrations, ROS levels, lipid peroxidation, antioxidative enzymes activities, reduced glutathione (GSH) and oxidized glutathione (GSSG) contents in ramie were determined. Assessing the effects of nanoparticles on plants under metal stress will provide new insights into the combined use of nanotechnology and phytoremediation in the remediation of polluted environment, as well as the potential risks of nanoparticles to plants under metal stress.

EXPERIMENTAL SECTION

Sediments Characterization. The preparation and characterization of sediments are presented in the Supporting Information (SI).

Nanoparticles Synthesis and Characterization. nZVI and S-nZVI were synthesized by the borohydride reduction method (laboratory preparation and characterization of nanomaterials are described in the SI).[38]

Plant Growth. Ramie seedlings were obtained from the Ramie Institute of Hunan agriculture university, China. Before transplanting, Cd-contaminated sediments were mixed with nZVI and S-nZVI thoroughly to obtain 0, 100, 500, and 1000 mg/kg nanoparticles contained Cd-contaminated sediments. Purified water was added to the sediments to maintain sediments moisture of 80% field capacity. Then ramie seedlings were transplanted into the above substrates and cultivated in a growth chamber at 14 h photoperiod (6:00 to 20:00), 24 ± 5 °C and 18 ± 3 °C day and night temperature, and 65 ± 5% relative humidity. The selected nanoparticles contents were based on the results from our pre-experiments, which showed that concentrations <100 mg/kg had little influence on ramie growth and development, while concentrations >1000 mg/kg significantly inhibited ramie seedlings growth.

Ramie seedlings were cultivated in the growth chamber for one month. Plants grown in the only Cd-contaminated sediments without nanoparticles were used as the control. All the treatments were performed in four replicates. After one month cultivation, plants were harvested. To determine the effects of nanoparticles on plant growth under metal stress, ramie leaves, stems and roots were collected separately and dried in oven at 70 °C until constant weight to record the biomass.

For nZVI treated plants, plant biomass and Cd concentration were determined.

Metal Analysis. To assess Cd and Fe contents and their translocation in plants, ramie leaves, stems and roots were harvested separately and washed with purified water thoroughly. For ramie roots, after washing with purified water, they were soaked in 10 mM Na_2EDTA (ethylenediaminetetraacetic acid disodium salt) for 15 min to remove metal ions (especially Cd and Fe ions) adhering to the root surface,[39,40] and then rinsed three times with purified water. All the washed tissues were dried until constant weight, and then digested with a mixture of 10 mL HNO_3 and 3 mL $HClO_4$ using a graphite digestion apparatus (DS-360). Cd and Fe contents in different plant tissues were analyzed by an atomic absorption spectrometer (AAS, PEAA700, PerkinElmer). The

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amount of Cd accumulated in each plant (A-Cd) and total Cd concentration (T-Cd) in ramie are calculated as follows:

$$A - Cd = Cd_L \times B_L + Cd_S \times B_S + Cd_R \times B_R \quad (4)$$

$$T - Cd = \frac{A - Cd}{B_L + B_S + B_R} \quad (5)$$

where Cd_L, Cd_S, and Cd_R are the concentration of Cd in leaves, stems and roots, respectively. B_L, B_S, and B_R represent ramie dry biomass in leaves, stems and roots, respectively.

Besides Cd and Fe contents, the subcellular distribution of Cd in different plant tissues was measured (detection method is described in the SI).

Oxidative Stress Estimation. H_2O_2 and Malondialdehyde (MDA) concentrations were detected following the published methods (detection methods are described in the SI).[37]

Antioxidants Contents Analysis. To investigate whether plant antioxidant systems under Cd-stress were modified by nanoparticles, antioxidant enzymes activities and specific antioxidants contents were measured. The activities of CAT, guaiacol peroxidase (POD), SOD, and the contents of GSH and GSSG in ramie seedlings were assayed using commercial reagent kits purchased from Nanjing JianCheng Bioengineering Institute, China (detection methods are described in the SI).

Statistical Analysis. The results are presented as means ± standard errors of 4 replicates. Data were analyzed using one-way Analysis of Variance (ANOVA), followed by Duncan test at a probability level of $P < 0.05$, performed by Statistic Package for Social Science.

RESULTS AND DISCUSSION

Materials Characterization. The characterization of nZVI and S-nZVI are described in the SI.

nZVI and S-nZVI Treatments. The effects of nZVI and S-nZVI on biomass production and Cd accumulation in ramie seedlings are presented in the SI.

Effects of S-nZVI on Seedlings Growth. To investigate the effects of nanoparticles on plants under metal stress, the plant growth of ramie was determined. Plant dry biomass of different tissues is shown in Figure 1. The dry weight decreased from 0.89 to 0.55 g/plant in leaves, from 1.38 to 1.14 g/plant in stems and from 2.02 to 1.53 g/plant in roots with increased S-nZVI concentrations, along with the dry biomass being 0.89, 1.44, and 2.04 g/plant in the leaves, stems and roots of plants cultivated in the only Cd-contaminated sediments, respectively. As shown in Figure 1, 100 and 500 mg/kg S-nZVI had no significant influence on plant growth under Cd stress, except in the roots exposed to 500 mg/kg S-nZVI, where there is a pronounced decrease of the dry weights (19.63%). Significant growth inhibition of ramie was observed at 1000 mg/kg S-nZVI treatment, indicating the nanotoxicity of S-nZVI and an aggravation of Cd toxicity in plants. As previously reported, high concentrations of nZVI are toxic to plants.[41] El-Temsah and Joner[42] found that nZVI at low concentration could be used without detrimental effects on plants, whereas the shoot growth reduction and seeds germination inhibition were observed in ryegrass, flax and barley exposed to high dosages of nZVI. Plant growth inhibition may be due to the coated nZVI on the root surface thus preventing nutrients uptake into plants.

Cd Accumulation and Translocation in S-nZVI Treated Seedlings. To investigate the effects of S-nZVI on Cd accumulation, Cd concentration in ramie leaves, stems, and roots were determined (Figure 2). As shown in Figure 2, Cd concentration in the control plants was 2.89, 3.49, and 7.83 mg/kg DW in the leaves, stems and roots, respectively. This is similar to what was observed in a previous study, wherein Cd content was 0.6 mg/kg in the shoots, and 5.5 mg/kg in the roots of *Acer pseudoplatanus* cultivated in 14.3 mg/kg Cd polluted soils.[43] The present results showed that Cd could be absorbed by ramie roots and translocated to the aboveground

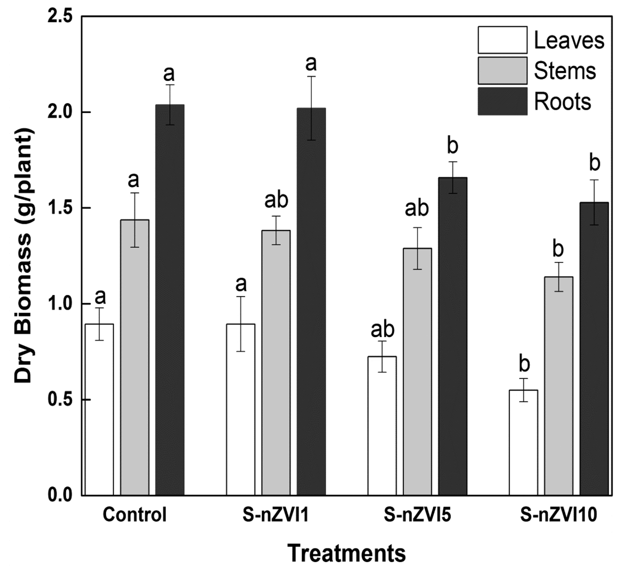

Figure 1. Dry biomass of the leaves, stems and roots of ramie seedlings treated with different concentrations of S-nZVI. Control, S-nZVI1, S-nZVI5, and S-nZVI10 denote 0, 100, 500, and 1000 mg/kg S-nZVI treatments. Data are means ± standard error of four replicates. Different letters (a, b, c) above the error bars indicate significant differences among different treatments ($p < 0.05$) using one-way ANOVA followed by Duncan test.

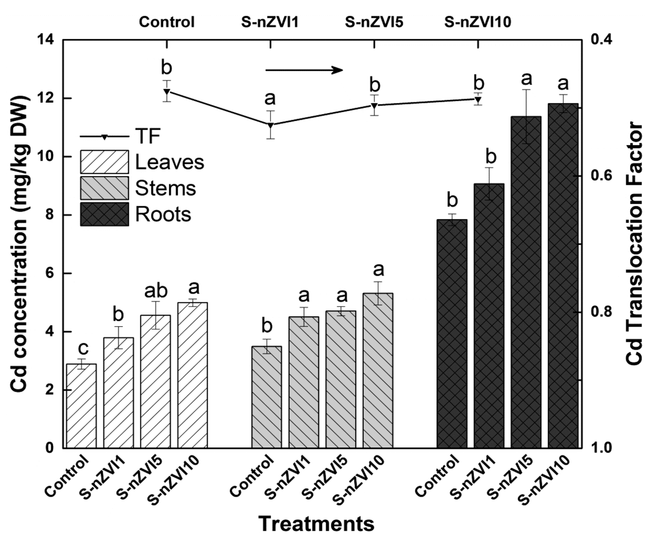

Figure 2. Cd accumulation and translocation in ramie seedlings treated with different concentrations of S-nZVI. Control, S-nZVI1, S-nZVI5, and S-nZVI10 denote 0, 100, 500, and 1000 mg/kg S-nZVI treatments. Data are means ± standard error of four replicates. Different letters (a, b, c) above the error bars indicate significant differences among different treatments ($p < 0.05$) using one-way ANOVA followed by Duncan test.

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parts, as reported previously.[44,45] Plants roots accumulated higher Cd when compared with the stems and leaves, which may be due to the protective reaction of the aboveground parts and the direct contact of roots with contaminant. Plants treated with S-nZVI had significantly more Cd than the control plants. Relative to the control, S-nZVI increased Cd concentration in ramie roots, stems and leaves by 16−50%, 29−52%, and 31−73%, respectively, suggesting that enhanced Cd accumulation by S-nZVI was more pronounced in plant aboveground parts. Cd concentration in ramie tissues increased with increasing S-nZVI application concentrations. The highest Cd content was observed in ramie treated with 1000 mg/kg S-nZVI. Because the accumulation of Cd in plants not only depends on the Cd content in different tissues but also relates to plant biomass, the A-Cd was calculated (SI Figure S5). Relative to the control plants, A-Cd in ramie treated with S-nZVI increased by 14−20%. The higher Cd accumulation in ramie was not found in the treatment of 1000 mg/kg S-nZVI, but was observed in the treatments of 100 and 500 mg/kg S-nZVI. Compared with the Cd accumulation in the control plants, Cd uptake in the whole plants was approximately 1.18 and 1.20 times higher in the 100 and 500 mg/kg S-nZVI treatments respectively, whereas plants treated with 1000 mg/kg S-nZVI only had a slight increase of the Cd accumulation. These results were likely due to the nanotoxicity of S-nZVI to plants at high concentrations, which inhibited plant growth, thus leading to a relative lower amount of Cd accumulated in the 1000 mg/kg S-nZVI treated plants compared with that in plants exposed to other concentrations of S-nZVI. The translocation of Cd in ramie was estimated using the translocation factor (TF), which is defined as the ratio of Cd in the aerial parts to that in roots. As shown in Figure 2, all the S-nZVI treatments, especially 100 mg/kg S-nZVI, increased the TF value of Cd in ramie seedlings. These results showed for the first time that nZVI promoted Cd accumulation and translocation in the plants. Similar to what was observed in our study, nanotitanium dioxide with the concentration range from 100 to 300 mg/kg was found to increase the uptake of Cd in *Glycine max* (L.) Merr.[46] However, different from what was observed in the present study, the accumulation of Cd and Cr in wheat plants exposed to iron-contained nanoparticles at concentration of 1000 mg/kg was considerably decreased.[47] Gil-Díaz et al.[26] also found that nZVI decreased the uptake and translocation of As in barley plants. Thus, it was speculated that the effects of nanoparticles on metal accumulation in plants depended on many factors, such as nanomaterials kinds, plant species, metal types and so forth.

Influence of S-nZVI on Cd Subcellular Distribution. In order to investigate the effects of S-nZVI on Cd uptake in ramie at cell level, the subcellular distribution of Cd in different plant tissues was measured. As shown in Figure 3, Cd was mainly distributed in the cell wall and soluble fraction with the proportion of Cd being 48−66% and 31−46%, respectively. These findings are consistent with previously published studies. For example, Ramos et al.[48] demonstrated that in the leaves cells of *Lactuca* sp., approximately 64% of Cd was in the cell wall fraction; over 70% of the total Cd was found in the soluble fraction of root cells in peanut exposed to 200 mM $CdCl_2$.[49] Plant cell wall acts the first barrier against entry of extracellular substances into the cell which is composed of polysaccharides, proteins, enzymes, and other molecules. The negative charge of cell wall and S-containing proteins and other function groups, such as amino group, hydroxyl and carboxyl, provide preferable conditions for Cd binding and detoxification, resulting in a large proportion of Cd in cell wall. Cell soluble fraction also occupied a majority percentage of Cd in ramie. This could be due to the vacuolar compartmentation of Cd in the cell soluble fraction. S-nZVI treated ramie seedlings showed a dose-dependent decrease in the proportion of Cd in cell wall compared with the control, whereas the accumulation of Cd in soluble fraction increased with increased S-nZVI concentrations. These results indicated that S-nZVI enhanced the penetration of Cd into plant cells. It seems that the increased Cd concentration in the soluble fraction of root cells resulted in the enhancement of Cd accumulation in plants. It has been reported that iron nanoparticles could trigger cell wall loosening, reduce cell wall thickness and enhance endocytosis in plants,[50] which could explain the promotion of Cd entering plant cells by S-nZVI. Moreover, the production of new pores and enlargement of existing pores in cell wall by nanoparticles have been confirmed by recent reports,[51] and these might account for the enhancement of Cd uptake and migration in plant cells exposed to S-nZVI. In addition, Cd concentration in the organelle fraction increased by S-nZVI, except in the 100 mg/kg S-nZVI treatment, where there was a slight decrease in the percentage of Cd in the organelle fraction of ramie roots and stems. The 100 mg/kg S-nZVI treatment significantly increased the T-Cd and A-Cd in ramie seedlings. However, the results on plant biomass production (Figure 1) revealed that 100 mg/kg S-nZVI did not cause statistically negative impact on ramie growth and development. This is probably due to the alleviation of Cd induced toxicity in plants by decreasing the proportion of Cd in the cell organelle fraction at 100 mg/kg S-nZVI treatments.

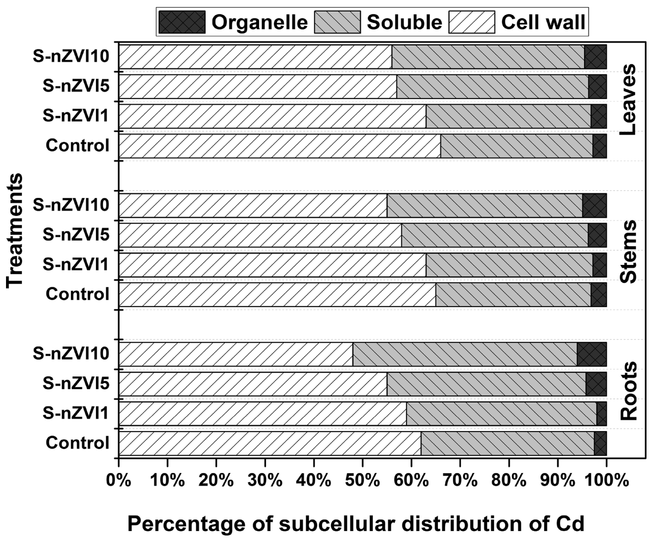

Figure 3. Subcellular distribution of Cd in the leaves, stems and roots of ramie seedlings treated with different concentrations of S-nZVI. Control, S-nZVI1, S-nZVI5, and S-nZVI10 denote 0, 100, 500, and 1000 mg/kg S-nZVI treatments.

Effects of S-nZVI on Fe Uptake. The uptake and translocation of nanoparticles in plants have been demonstrated in a variety of reports, and this may provide an effective means of increasing metal accumulation in plants. For a better assessment of the effects of S-nZVI on Cd accumulation, Fe content in different plant tissues was measured. Since Fe is an essential nutrient, some Fe was observed in the different plant tissues. As shown in Figure 4, S-nZVI increased Fe content in ramie tissues and this enhancement was more remarkable as S-

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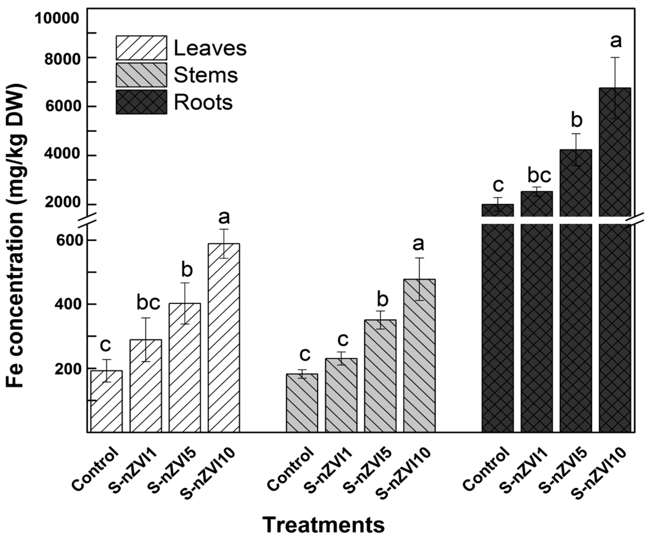

Figure 4. Fe uptake in the leaves, stems and roots of ramie seedlings treated with different concentrations of S-nZVI. Control, S-nZVI1, S-nZVI5, and S-nZVI10 denote 0, 100, 500, and 1000 mg/kg S-nZVI treatments. Data are means ± standard error of four replicates. Different letters (a, b, c) above the error bars indicate significant differences in the accumulation of Fe in leaves, stems and roots, respectively, among the treatments ($p < 0.05$).

nZVI concentrations increased. Although the speciation of Fe in plant tissues was not measured, previously published studies have confirmed that nZVI could be absorbed by plants. Ma et al.[28] reported that nZVI could adhere on root surface and penetrate into epidermal cells of hybrid poplars. The existence of nZVI in *Sinapis alba* and *Sorghum saccharatum* has been observed by bright-field micrographs and DIC images, which certified the uptake of nZVI into plant seedlings.[29] The chemical transformation of S-nZVI in ramie was not determined in this study and has not yet been reported. However, according to the increased uptake of Fe observed in ramie seedlings exposed to S-nZVI and some previous investigations, it is hypothesized that S-nZVI could be absorbed by ramie seedlings. In addition, S-nZVI treatments increased Fe content in leaves by 51−207% and in stems by 27−162%, respectively. It is expected that S-nZVI could penetrate the root surface and some of them were likely to be transported to the aboveground parts of plants. Further studies are required to determine the speciation of Fe and the biotransformation of iron nanomaterials in plants.

nZVI has been demonstrated to immobilize Cd through absorption and surface complexation.[52] In the present study, Cd accumulation and Fe uptake in ramie seedlings for all the S-nZVI treatments were quite similar. Therefore, we hypothesized that S-nZVI might serve as a Cd carrier to promote Cd uptake into plant seedlings. As reported by Tang et al., internalization of graphene oxide (GO) occurred in *Microcystis aeruginosa*, and the absorbed Cd on GO could easily enter this algae cells, thus increasing Cd uptake into plants.[19] Besides, Cd is a nonessential metal and it has no beneficial effects to plant growth and development to our knowledge. As reported previously, there are no confirmed specific transport channels for Cd in plants. Cd was demonstrated to get access into plants through the channels and transporters for essential elements, such as calcium, manganese and potassium.[53−55] Specifically, Connolly et al. found that the overexpression of IRT1, a Fe transporter, in *Arabidopsis thaliana* resulted in a higher level of Cd accumulation.[56] In the present work, the addition of S-nZVI to the contaminated sediments probably will stimulate the channels and transporters for Fe uptake, which sequentially give rise to the promotion of Cd accumulation in the plants.

In the present study, results showed that Fe content in ramie seedlings increased as the external S-nZVI concentrations increased. However, relative to the control, 1000 mg/kg S-nZVI did not show statistically significant increase of A-Cd in ramie seedlings (SI Figure S5). Similar to previous reports in sunflower plants grown in Cd-polluted soil, Fe content was by far greater in the plants exposed to *Streptomyces tendae* F4, whereas Cd accumulation in these plants only increased slightly.[57] In addition, previous investigations revealed that Fe at relatively high level suppressed Cd uptake in plants.[58] These results indicate that the influences of Fe on Cd uptake are largely concentration-dependent. Considering the Fe content in plants was changed by the external S-nZVI concentrations, the effects of S-nZVI on metal accumulation are concentration-dependent.

However, there were no statistical differences in Fe content between the plants treated with 100 mg/kg S-nZVI and the control plants. Since plants exposed to 100 mg/kg S-nZVI accumulated significantly more Cd in ramie compared to the control, the result indicate that enhanced uptake of nanoparticles is only partly the cause of the promoted Cd accumulation. Further studies are needed to explore the exact mechanisms by which nanoparticles affect heavy metals uptake and translocation in the plants.

Influence of S-nZVI on Oxidative Stress. To evaluate the effects of nZVI on the oxidative damage in plants under Cd stress, the concentrations of MDA and H_2O_2 in ramie seedlings were measured. MDA is a product of peroxidation processes, which can react with DNA and is mutagenic to organisms.[59] MDA can be typically used as the indicator of lipid peroxidation in plants, which has been certified by many previous studies.[60,61] H_2O_2 is a representative ROS, and its excessive accumulation indicates oxidative damage in plants. The overproduction of H_2O_2 can cause oxidative injury to proteins, DNA, and lipids.[62] As shown in SI Figure S6, 500 and 1000 mg/kg S-nZVI increased MDA concentration by 30−44%, whereas the 100 mg/kg S-nZVI did not significantly change the MDA content. The highest (292.22 nmol/g FW) and the lowest (123.35 nmol/g FW) H_2O_2 content were observed in ramie seedlings treated with 1000 and 100 mg/kg S-nZVI (SI Figure S7), respectively. The responses of MDA and H_2O_2 to the 500 and 1000 mg/kg S-nZVI followed the same pattern: significantly increased, relative to the non-nanomaterial treatment. Since MDA and H_2O_2 are indicators of oxidative stress in plants, the results indicate that some concentrations of S-nZVI aggravated the oxidative damage in ramie under Cd stress which may be due to the increased uptake of Cd in these treatments. However, there were no significant differences in Cd accumulation between the 500 and 1000 mg/kg S-nZVI treatments, but H_2O_2 content significantly increased in ramie exposed to 1000 mg/kg S-nZVI and a slight increase in MDA was also observed in this treatment. Therefore, we hypothesized that the aggravated oxidative stress at high level of nanoparticles treatments was due to the promoted Cd uptake and the nanotoxicity of nZVI. In the presence of oxygen, nZVI has H_2O_2 production ability (reaction 1).[63] Kim et al.[50] demonstrated that nZVI promoted H_2O_2 generation and release in the plant medium. The oxidative stress was observed in human bronchial epithelial cells exposed to nZVI.[64] It is

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hypothesized that nZVI might induce oxidative damage to plants through ROS generation or conversion (reaction 1−3).[59] Specifically, the increase of MDA in ramie seedlings treated with higher level of S-nZVI indicate the aggravation of lipid peroxidation in plants, which is probably due to the attack of nZVI on proteins and polyunsaturated fatty acid residues in phospholipids.[65] Moreover, among all the treatments, H_2O_2 and MDA contents were the lowest in the 100 mg/kg S-nZVI treatment, indicating that S-nZVI could alleviate Cd-induced oxidative stress in ramie under certain conditions. The different results obtained in all the treatments suggest that the effects of nanoparticles on plant oxidative stress depend on their concentrations. These results are in agreements with those observed in rice seedlings, wherein cerium oxide nanoparticles at 62.5 and 125 mg/L significantly reduced H_2O_2 production and 500 mg/L nanoparticles enhanced the lipid peroxidation and the electrolyte leakage.[66] In addition, the aggravated oxidative damage by S-nZVI at high concentrations could have resulted in the growth inhibition of ramie exposed to Cd.

Effects of S-nZVI on the Antioxidant Defense System. Plants have evolved well-equipped strategies consisting of different enzymes, such as SOD, CAT and POD, to fight against oxidative damage. SOD catalyzes the reaction of $O_2{\bullet}^-$ to H_2O_2 while CAT and POD promote the conversion of H_2O_2 to H_2O. The changes of these antioxidative enzymes activities in ramie by S-nZVI under Cd stress are shown in Figure 5A. Relative to the control, antioxidative enzymes activities in ramie treated with 100 mg/kg S-nZVI increased. The responses of SOD and POD in ramie exposed to 500 mg/kg S-nZVI followed similar pattern: significantly decreased, compared with the control. The decrease was more remarkable at 1000 mg/kg S-nZVI treatments. CAT activity did not change significantly by S-nZVI, except in the 1000 mg/kg S-nZVI treatment, wherein CAT activity decreased by 21% compared with the control.

Nonenzymatic antioxidative systems composed of antioxidants also perform roles in the scavenging of overproduced ROS. GSH is a low molecular weight antioxidant which is capable of scavenging ROS. In the ascorbate-glutathione cycle, H_2O_2 is reduced to water accompanied by the transformation of GSH to GSSG. The ratio of GSSH/GSH is the indicator of cellular redox status. In the present study, GSH and GSSG were differentially affected by the concentrations of S-nZVI (Figure 5B). Compared with the control, 100 mg/kg S-nZVI increased GSH content by 17%, while the 500 and 1000 mg/kg S-nZVI significantly reduced its concentration in ramie seedlings. On the contrary, GSSH content was the lowest in the 100 mg/kg S-nZVI treatment, and it increased remarkably with increased S-nZVI concentrations. As for the ratio of GSSG/GSH, the 500 and 1000 mg/kg S-nZVI increased it by 90% and 160%, respectively, whereas the 100 mg/kg S-nZVI did not significantly change this ratio in ramie seedlings.

The different antioxidant responses in ramie seedlings exposed to S-nZVI suggest that nZVI can modify the antioxidative defense systems in plants. The increased activities of SOD, POD, and CAT, and the enhanced content of GSH in ramie seedlings treated with 100 mg/kg S-nZVI suggest that S-nZVI could improve the antioxidative capacity of ramie under Cd-stress at this concentration. Fe is a cofactor or constituent for many enzymes, such as CAT, ascorbate peroxidase and GSH peroxidase.[67] Specifically, Fe is required as the metal active site for one type of SOD (Fe-SOD), and it can modify the activities of many antioxidative enzymes in plants under metal stress.[68] For example, a study conducted by Liu et al.

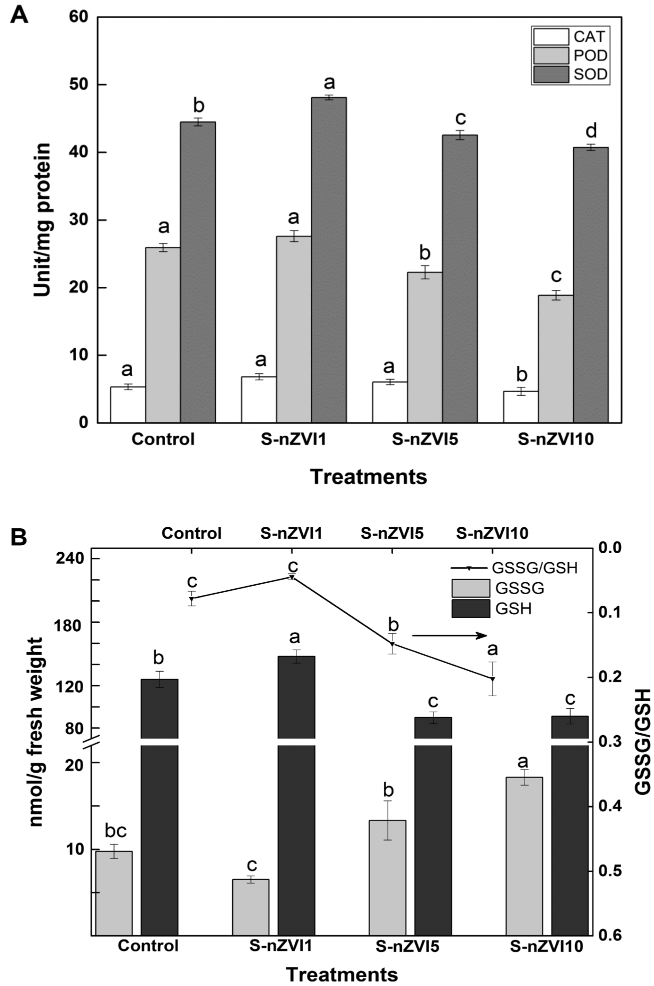

Figure 5. Antioxidant substances concentrations in ramie seedlings treated with different concentrations of S-nZVI. A corresponds to the activities of CAT, SOD, and POD in ramie seedlings. B corresponds to the contents of GSSH and GSH, and the ratio of GSSG/GSH in ramie seedlings. Control, S-nZVI1, S-nZVI5, and S-nZVI10 denote 0, 100, 500, and 1000 mg/kg S-nZVI treatments. Data are means ± standard error of four replicates. Different letters (a, b, c) above the error bars indicate significant differences among different treatments ($p < 0.05$) using one-way ANOVA followed by Duncan test.

showed that the interaction between Fe and Cd increased the activities of SOD and CAT in rice.[69] It seems that the increased Fe content at 100 mg/kg S-nZVI treatment result in the enhanced activities of SOD, POD and CAT in the present study. As mentioned above, H_2O_2 content in ramie treated with 100 mg/kg S-nZVI was remarkable lower compared with the control. Thus, this could have resulted from the changes in the antioxidative enzymes activities and antioxidant contents of ramie treated with this concentration of S-nZVI. These findings are consistent with previous reports. Wu et al.[70] reported that Ni/Fe nanoparticles reduced the oxidative damage to Chinese cabbage cultivated in polybrominated diphenyl ethers contaminated soils through changing the activities of SOD, CAT, and POD. Tripathi et al.[71] found that in *Pisum sativum* (L.) seedlings, silicon nanoparticles protected plants against Cr(VI) phytotoxicity via up-regulating the antioxidant defense systems. Therefore, we hypothesized that low level of iron nanoparticles (≤100 mg/kg) could counteract metal-induced oxidative stress in plants via improving the activities of antioxidative enzymes

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and enhancing the concentrations of some antioxidants. On the other hand, the increased GSSH content and the raised ratio of GSSG/GSH in ramie seedlings exposed to higher level of S-nZVI indicated a gradual shift to more oxidized cellular redox status in plants. These changes were in accordance with the increased contents of H_2O_2 and MDA. The over production of ROS in plants could lead to an increase in the activities of antioxidative enzymes and the contents of antioxidants; however, the results were contrary to the expectation in our experiments. The decreased activities of SOD, CAT and POD and the reduced content of GSH in higher level of S-nZVI treatments presumably were because of the phytotoxicity of nanoparticles. As indicated by Hong et al.,[72] nano ceria caused toxicity in *Cucumis sativus* by modifying the activities of CAT, ascorbate peroxidase and dehydroascorbate reductase; silver nanoparticles induced oxidative stress in *Triticum aestivum* L. by the accumulation of GSSG.[73] In addition, the promoted Cd uptake in plants treated with 500 and 1000 mg/kg S-nZVI perhaps also account for the decreased antioxidative enzymes activities. Overall, higher level of S-nZVI blocked the antioxidative defense of plants under Cd-stress by enhancing antioxidative enzymes activities and reducing antioxidant concentrations.

In summary, the findings of the present study demonstrate that S-nZVI promote the uptake and translocation of Cd in ramie seedlings. Moreover, the oxidative stress studies demonstrate that S-nZVI can modify the oxidative damage and the antioxidative responses of plants under Cd-stress. High contents of S-nZVI aggravated the oxidative damage in plants under Cd-stress resulting from the promoted Cd accumulation and the phytotoxicity of nanoparticles. Although 100 mg/kg S-nZVI promoted Cd uptake, it alleviated Cd-induced oxidative damage in plants by scavenging ROS, increasing antioxidative enzymes activities and enhancing the antioxidant contents. These results suggest that proper concentrations of nanoparticles are beneficial to promote Cd accumulation and alleviate metal-induced toxicity in plants. Therefore, the combined utilization of nanotechnology and phytoremediation can be regarded as a promising feasible technique for the remediation of metal contaminated environment. While the application of nanoparticles to facilitate soil phytoremediation is only starting to be realized, further studies on the mechanisms of metal uptake and translocation, as well as plant toxicity influenced by nanoparticles, are needed.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b03164.

Supplemental methods, additional results and discussion, table, and figures as mentioned in the text (PDF)

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Notes

The authors declare no competing financial interest.

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