Bull Environ Contam Toxicol (2009) 82:338–342
DOI 10.1007/s00128-008-9573-8

Iron-Deficiency Induces Cadmium Uptake and Accumulation in *Solanum nigrum* L.

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Received: 20 December 2007 / Accepted: 2 October 2008 / Published online: 11 November 2008

Abstract Phytoremediation is a promising tool in removing pollutants from the environment or in rendering them harmless. The objective of this research was to determine the effect of iron-deficiency on the uptake of cadmium by *Solanum nigrum* L. Results showed that iron-deficiency induced cadmium uptake, biomass decrease and changes in pH and Eh in hydroponic culture. Under iron-deficiency status, the decrease in pH and the increase in Eh might result in higher cadmium availability. Bioconcentration and translocation factors indicated that iron-deficiency status affected cadmium accumulation and translocation in *Solanum nigrum* L.

Keywords Phytoextraction · Iron-deficiency · *Solanum nigrum* L. · Cadmium

Heavy metals are widespread and persistent environmental pollutants that are released into the environment by anthropogenic activities, such as mining, smelters, power station industry, and the application of metal-containing pesticides, fertilizers, and sewage sludge (Malik 2004). Phytoremediation is defined as the use of green plants in removing pollutants from the environment or in rendering them harmless (Salt et al. 1998). Phytoremediation is currently being considered as a promising, cost-effective, aesthetically pleasing technology for the remediation of polluted sites and requires smaller disposal facilities. Plants with enhanced iron (Fe) acquisition and storage strategies can help us to improve human health through balanced mineral nutrition; on the other hand, they may help us to remediate contaminated soil through the absorption of heavy metals. Cohen et al. (1998) reported that Fe deficiency induced an increased capacity to absorb Fe and other micronutrient and heavy metals such as Zn^{2+} and Cd^{2+} into pea (*Pisum sativum* L.) roots.

Solanum nigrum L. species are worldwide weeds of arable land, but in many developing countries they constitute a minor food crop, with the shoots and berries not only being used as vegetables and fruits, but also for various medical and local uses. A cadmium-hyperaccumulator *Solanum nigrum* L. species was first discovered in heavy metal contaminated areas (Wei et al. 2005). Thus, *Solanum nigrum* L. plants growing in heavy-metal polluted soils can be a risk via the food chain and feed chain for human health.

The present research was conducted to evaluate the effect of plant Fe deficiency on cadmium (Cd) uptake by *Solanum nigrum* L. in hydroponic culture, in order to obtain novel information on the phytoremediation of this toxic metal.

Materials and Methods

Seeds of tested plant (*Solanum nigrum* L.) were obtained from Shenyang University Key Laboratory of

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Environmental Engineering. The seeds were sterilized in 75% ethanol for 10 min and washed several times with sterile distilled water. The seeds were dipped in sterile distilled water and shaken at 144 r/min on an orbital shaker in sterile distilled water for 8 h. Then the seeds were germinated on filter paper moistened with distilled water in a thermostat for 2 days. Temperature and relative humidity were kept on 28°C and 60%, respectively. After 1 week of incubation, seedlings with similar biomass were transferred to hydroponic culture under sterile conditions. Plants were grown in a controlled-environment growth chamber with a 16 h light period (light intensity of 350 μmol m^{-2} s^{-1}), a 25/15°C light/dark temperature regime, and 60% relative humidity. Plants were harvested after 5 weeks growth.

Plants were grown hydroponically to study their ability to accumulate and tolerate different concentrations of Cd under Fe-deficiency. Seedlings of plants were placed through a perforation in a plastic platform in a 450-mL plastic jar containing 400 mL of Hoagland's solution (Hoagland and Arnon 1938), so that the root was immersed in liquid medium and the shoot was above the platform. Sterility checks were conducted in preparation cultures simultaneously. The heavy metal salt used in this study was $CdCl_2 \cdot 2.5H_2O$ (analytical purity). The salt was diluted in deionized water and added into the hydroponic cultures. Treatments were prepared at cadmium concentrations of 0 mg/L (control), 1 mg/L, 5 mg/L, 10 mg/L, 20 mg/L. All solutions were adjusted to pH 6.0–6.2.

Plants were grown in quarter-strength Hoagland's solution in the first week, and then cultures were changed to half-strength Hoagland's solution in the second week. During the next 2 weeks the cultures were changed to full strength Hoagland's solution. In the fifth week, some seedlings were still in full strength Hoagland's solution (+Fe), the others were generated in full strength Hoagland's solutions without Fe (−Fe). After the seedlings had grown for 5 weeks, seedlings were exposed to the different Cd concentrations for 4 days. Plants were arranged in a completely randomized design. During 5 weeks, cultures were continuously aerated with an aquarium air pump, replaced with fresh solution every 2–3 day and supplied deionized water to maintain 400 mL in all treatments.

During harvest, the plants were rinsed with distilled water and shoot and root were separated. The plant samples were oven dried at 70°C for 48 h to a constant weight, after which dry weight of shoots and roots was determined by electronic balance. The plant tissues were ashed at 500°C for 5 h in a muffle furnace and cooled down. A mixture of 5 mL HNO_3 (65%), 2 mL H_2O_2 (30%) and 2 mL purified water (Milli-Q reagent grade water) were added at room temperature. Subsequently, volume of samples was adjusted to 20 mL with deionized water and analyzed for cadmium by flame atomic absorption spectroscopy (Spectra AA220, Varian). A certified reference material (bush leaf material) GBW07603, Qunghai Province, China was used to monitor the recovery of metals from the plant samples. Values of pH and Eh in hydroponic cultures of treatments were analyzed using a platinum electrode and glass electrode. To evaluate the phytoextraction potential, two equations were used. (1) the bioconcentration factor ($BCF = C_{plants}/C_{culture}$) and (2) the translocation factor ($TF = C_{shoots}/C_{roots}$).

Controls and treatments were performed in triplicates. Data were tested for statistical significance using one-way ANOVA by SPSS11.0 software package, followed by the least significant difference (LSD) test for comparison of individual means. The difference was considered significant at $p < 0.05$.

Results and Discussion

The results showed that *S. nigrum* growing in the solution with Fe had the highest biomass (0.67 g) in the Cd 0 mg/L treatment ($p < 0.05$), while the biomass of *S. nigrum* growing without Fe (0.22 g) was the smallest in the Cd 20 mg/L treatment ($p < 0.05$) (Fig. 1). In the treatments of different cadmium concentrations, the biomass of *S. nigrum* plants grown without Fe was less than the plants grown with Fe ($p < 0.05$), which showed that Fe was a very important element for plant growth. The biomass of *S. nigrum* plants decreased with increasing Cd concentrations whether Fe was supplied or not to the plants. Generally speaking, Fe-deficiency can affect photosynthesis and decrease dry matter production. Thus, the biomass of the Fe-deficient plants was lower than the Fe-sufficient

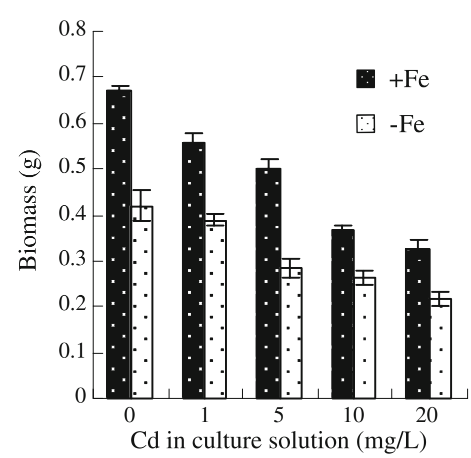

Fig. 1 Effect of Fe-deficiency on biomass by *Solanum nigrum* at different Cd concentrations. −Fe and +Fe represent the solution without Fe and with Fe. Vertical bars represent ± SE of triplicates (n = 3)

plants in this experiment, which might be a result of Cd toxicity or Fe deficiency.

As presented in Fig. 2, the content of Cd in the Fe-sufficient *S. nigrum* plants grown with Cd 20 mg/L was the highest (147.67 mg kg^{-1}) ($p < 0.05$); and the content of Cd was the lowest (38.56 mg kg^{-1}) ($p < 0.05$) in the Fe-sufficient *S. nigrum* plant grown with Cd 1 mg/L. Fe-deficient *S. nigrum* seedlings accumulated more cadmium at 1, 5 and 10 mg/L cadmium concentrations compared to Fe-sufficient seedlings. In this experiment, Fe deficiency contributed to an increase in cadmium accumulation. This may be explained that Fe deficiency induced the activity of a plasma membrane ferric chelate reductase and also stimulated the release of protons, both of which enhanced the solubility of cadmium. Rodecap et al. (1994) reported that Fe-deficient Arabidopsis plants accumulated higher concentrations of Cd and Mg in racemes and seeds compared to Fe-sufficient plants. Cohen et al. (1998) demonstrated that Fe deficiency induced a high Cd influx into roots of pea seedlings, a finding that has implications for the entry of heavy metals into food crops. To explore the molecular basis, Cohen et al. (2004) found that Fe deficiency induced a divalent cation transporter that can facilitate Cd^{2+} influx.

Values of pH and Eh were shown in Fig. 3 and Fig. 4. pH values in the culture solution with and without Fe in concentrations of Cd ranged from 5.16 to 6.21. The highest pH appeared in the treatment of Cd 0 mg/L solution with Fe by *S. nigrum* ($p < 0.05$); the lowest pH appeared in Cd 5 mg/L solution without Fe by *S. nigrum* ($p < 0.05$). The pH of Fe-deficient *S. nigrum* culture solutions was lower at 0, 1, 5, 10 and 20 mg/L concentrations compared to the culture solutions of Fe-sufficient *S. nigrum* ($p < 0.05$). As presented in Fig. 6, the values of Eh in culture solution with Fe and without Fe in treatments of Cd concentrations

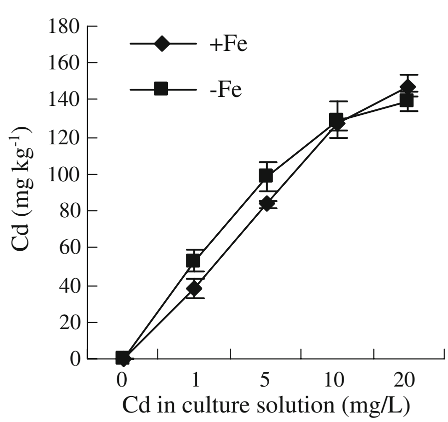

Fig. 2 Effect of Fe-deficiency on Cd bioaccumulation by *Solanum nigrum* at different Cd concentrations. −Fe and +Fe represent the solution without Fe and with Fe. Vertical bars represent ± SE of triplicates (n = 3)

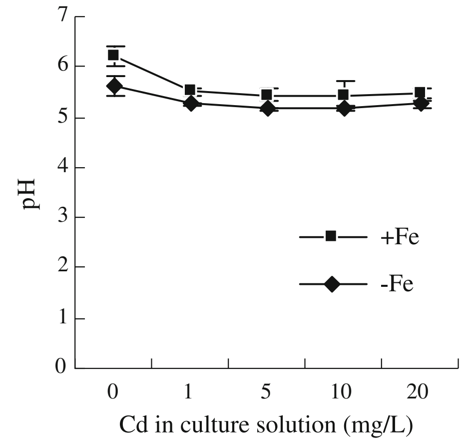

Fig. 3 Effect of Fe-deficiency on pH of solution by *Solanum nigrum* at different Cd concentrations. −Fe and +Fe represent the solution without Fe and with Fe. Vertical bars represent ± SE of triplicates (n = 3)

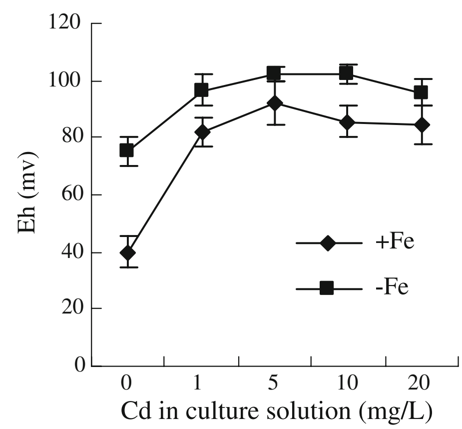

Fig. 4 Effect of Fe-deficiency on Eh of solution by *Solanum nigrum* at different Cd concentrations. −Fe and +Fe represent the solution without Fe and with Fe. Vertical bars represent ± SE of triplicates (n = 3)

fluctuated from 40 mv to 102.3 mv. The highest Eh appeared in the treatment of Cd 5 mg/L with Fe-deficient *S. nigrum* ($p < 0.05$); the lowest Eh appeared in Cd 0 mg/L treated with Fe-sufficient *S. nigrum* ($p < 0.05$).

The bioavailability of heavy metals in soil is influenced by many factors, such as cation exchange capacity, organic matter content and especially, pH which has been regarded as a master variable regulating the mobility of metals (Lim et al. 2002). Under Fe deficiency, species of Strategy I plants stimulate the release of protons, which could enhance the solubility of soil Fe. Redox status could affect the behavior of heavy metals or change the forms of heavy metals (Shuman and Wang 1997). Chuan et al. (1996) observed that metal solubilities increased as the redox potential decreased at the same pH value. Andrade et al.

(2004) reported that the lowering of Eh would have contributed to these heavy metals being found almost exclusively in insoluble forms, through the study of Cr, Cu, Ni, Pb, and V in Galician coastal sediments.

Translocation factor (TF) were tested in Fig. 5 and Fig. 6. TF values of Cd in culture solution with and without Fe ranged from 0.44 to 1.06. The highest TF occurred in the treatment Cd 1 mg/L by Fe-deficient *S. nigrum* ($p < 0.05$); the lowest TF appeared in Cd 20 mg/L treatment with Fe-deficient *S. nigrum* ($p < 0.05$). As presented in Fig. 7, BCF values of *S. nigrum* decreased with rising cadmium concentrations with and without Fe in the culture solution. BCF value of Fe-deficient *S. nigrum* was the highest (53) in Cd 1 mg/L treatment ($p < 0.05$), and the lowest BCF (6.97) appeared in Cd 20 mg/L treatment with Fe-deficient *S. nigrum* ($p < 0.05$).

Values of BCF can be an important index to estimate a plant's ability of assimilating and concentrating heavy metals from the root system to the aerial parts. Generally,

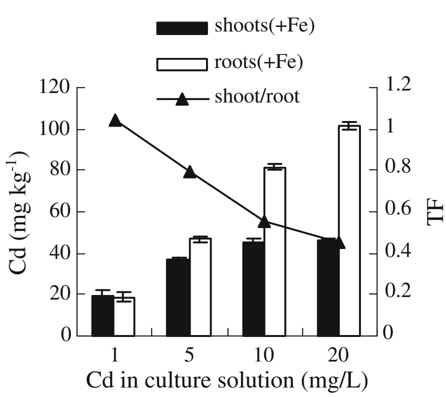

Fig. 5 Concentrations of Cd in the shoot and root tissue of *Solanum nigrum* grown in the solution with Fe (+Fe) at different Cd concentrations. Vertical bars represent ± SE of triplicates (n = 3)

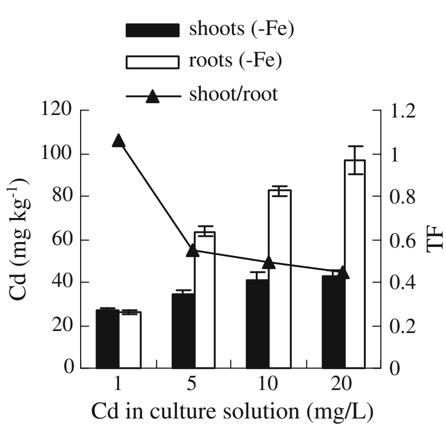

Fig. 6 Concentrations of Cd in the shoot and root tissue of *Solanum nigrum* grown in the solution without Fe (−Fe) at different Cd concentrations. Vertical bars represent ± SE of triplicates (n = 3)

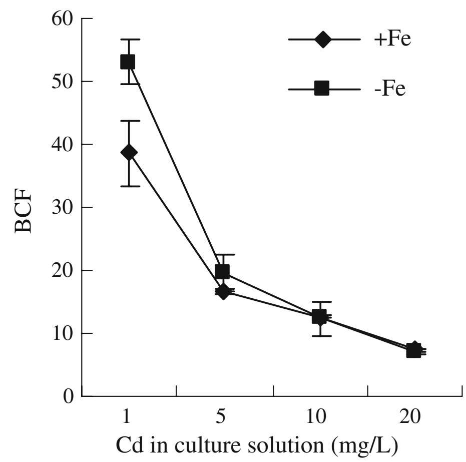

Fig. 7 Effect of Fe-deficiency on BCF of *Solanum nigrum* at different Cd concentrations. −Fe and +Fe represent the solution without Fe and with Fe. Vertical bars represent ± SE of triplicates (n = 3)

tissue concentrations of Cd tended to be higher in higher cadmium concentration solutions but BCF values were lower, suggesting that the transfer of cadmium from solution to the plant was less efficient at high cadmium concentrations. From BCF values, Fe-deficiency could affect the phytoextraction of cadmium by *S. nigrum*. The results indicated that the accumulation of cadmium was promoted when Fe was not added in solution, this may be due to Fe-deficiency made plant root excrete organic acids and other compounds which could change condition of redox and pH. For example, organic ligands could induce changes in soil pH and Eh, solubilization of solid bound Cd may take place as pH and Eh decreased and increased respectively (Collins et al. 2003). Values of TF can show the translocation of heavy metals from plant roots to shoots. Even though the *S. nigrum* accumulated more heavy metal, we could find the cadmium mainly accumulated in roots. But at Cd 1 mg/L concentration, the TF of *S. nigrum* with Fe and without Fe was 1.04 and 1.06 respectively, indicating that they had better ability to translocate cadmium from roots to shoots.

The findings of this study indicated that Fe deficiency promoted cadmium uptake through solution pH and Eh regulations. From the results of BCF and TF, Fe deficiency induced cadmium accumulation and accelerated the cadmium translocation efficiency. This research offered some useful information for phytoremediation.

Acknowledgments The author acknowledges the financial support from the National Basic Research Program of China (No. 2004CB418506); the National Natural Science Foundation (No. 200477029); the Science and Technology Bureau Program of Shenyang (No. 1071149-3-00); and the Open Foundation of Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences.

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