Zero-valent iron reduces cadmium and arsenic accumulation in spinach (*Spinacia oleracea* L.)

Takashi Nakamura [a], Mitsuru Osaki [a], Toshihiro Watanabe [a]

[a] *Research Faculty of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kitaku, Sapporo 0608589, Japan.*

Corresponding author: Toshihiro Watanabe (nabe@agr.hokudai.ac.jp)

Abstract

Cadmium (Cd) and arsenic (As) soil contamination poses significant problems for crop production worldwide. Zero-valent iron (Fe^0) is a reactive material with reducing power capable of stabilizing toxic elements, including heavy metals and metalloids. In this study, we examined the effect of Fe^0 application on Cd and As accumulation in spinach (*Spinacia oleracea* L.) grown in Cd- or As-contaminated soil. Fe^0 soil application reduced Cd and As concentrations in spinach shoots and/or roots, and decreased their availability in soil, which may have affected their accumulation in spinach. Additionally, we examined the forms of soil Cd and As using the sequential extraction method. The potential availability of Cd and As forms in soils for plant root is: exchangeable > iron-manganese-oxide-bound > organic-matter-bound > free-oxide-occluded > residual. Fe^0 application increased Cd and As distribution in the free-oxide-occluded fraction. Additionally, Fe^0 application decreased As distribution in the iron-manganese-oxide-bound fraction while tended to decrease Cd in the organic-matter-bound fraction and increase it in the iron-manganese-oxide-bound fraction. Thus, Fe^0 application can be effective in reducing Cd and As availability in their contaminated soils. Meanwhile, this study showed that Fe^0 application inhibited the growth of spinach in Cd-contaminated soil. The unfavorable alterations caused by Fe^0 in the contaminated soil should also be investigated in the future.

Keywords: arsenic, cadmium, sequential extraction method, spinach, zero-valent iron.

Introduction

Soil contamination by cadmium (Cd) and arsenic (As) is a significant agricultural problem worldwide (Arao et al. 2010; Bramley 1990; Rahaman et al. 2013). Crops cultivated in the contaminated soil often contain significant levels of Cd and As in their edible parts that can impair human health (Clemens and Ma 2016). Cadmium causes dysfunction of various organs and has a variety of adverse effects on human health (Huang et al. 2017). Chronic exposure to As increases the risk of diabetes, adverse pregnancy outcomes (APO), and cancer (Upadhyay et al. 2019). Therefore, reducing accumulation of Cd and As in crops is an important food safety issue (Dheri, Singh Brar, and Malhi 2007; Rahaman et al. 2013). Various remediation methods have been developed for soils contaminated with toxic elements. For example, phytoremediation reduces the concentration of toxic elements in the soil by allowing plants to absorb them (Alkorta et al. 2004), whereas chemical immobilization inactivates (insolubilizes) toxic elements in the soil rather than removing them (Liu et al. 2018). Zero-valent iron (Fe^0) is a reactive material with reducing power that can immobilize heavy metals and metalloids in water and soils (Lien and Wilkin 2005; Rangsivek and Jekel 2005; Hanauer et al. 2011). Xue et al. (2018) reported that Fe^0 interactions with toxic elements (heavy metals and metalloids) in the soil could be divided into five processes: reduction, adsorption, oxidation, co-precipitation, and precipitation and immobilization mechanism can be through one or more of the above processes.

In a previous study, we showed that Fe^0 application in flooded Cd-contaminated soil decreased its availability and reduced Cd concentration in rice leaves and grains (Watanabe et al. 2009). Hashimoto and Yamaguchi (2013) suggested that the decrease in Cd availability is due to enhanced CdS formation and reduced Cd in the exchangeable fraction and the soil solution due to Fe^0 application. Mlangeni et al. (2020) reported that the effect of Fe^0 soil application on Cd and As accumulation in rice varied with soil water management. Thus, there are several reports on the effects of Fe^0 soil application on toxic-element accumulation in rice under flooded conditions, but few studies on its effects in crops under non-flooded (upland) conditions.

Spinach (*Spinacia oleracea* L.) is a popular leafy vegetable with high nutritional value that is consumed worldwide (Roberts and Moreau 2016). However, spinach accumulates relatively high Cd and As concentrations in its leaves (Alexander, Alloway, and Dourado 2006; Bergqvist et al. 2014). In particular, concerning Cd, spinach tends to have a higher Cd content in its leaves than other leafy vegetables (John 1973). Therefore, reduction of Cd accumulation in spinach is a global concern (Abedi-Koupai, Mollaei, and Eslamian 2015; Sato et al. 2010). While the effects of Fe^0 on the uptake of Cd and As in spinach have not been fully investigated, it is expected that its reducing power and adsorption capacity of oxidized Fe in soils could potentially reduce the uptake of Cd and As in spinach. In this study, therefore, we examined the effect of Fe^0 application on Cd and As accumulation in spinach grown in Cd- and As-contaminated soils and estimated the various forms of soil Cd and As using the sequential extraction method.

Materials and methods

Preparation of Cd- and As-contaminated soils

The soil used for this study was collected from the plow layer (0−10 cm) of an experimental field at the Hokkaido University, Hokkaido, Japan. It was classified as a brown lowland type (haplic fluvisol), the general properties of which were shown in our previous report (Watanabe et al. 2016). Cd- and As-contaminated soils were prepared by adding 3 g of $CdCl_2$ and 10g of Na_2HAsO_4 to 30 kg of the sieved (2-mm mesh) field soils, respectively, and incubated in a greenhouse for one month with occasional watering. After the soil incubation, the concentrations of Cd (0.1 M HCl extractable) and As (1.0 M HCl extractable) in the soil were measured. The deficient amounts were then replenished, and the soils were incubated as in the first incubation. Finally, Cd- and As-contaminated soils were prepared as containing 50 mg kg^{-1} Cd and 100 mg kg^{-1} As, respectively. For Cd contaminated soil treatment, the soil was diluted with the sieved field soil to concentrations of 16 mg kg^{-1} Cd (0.1 M HCl extractable). For As contaminated soil treatment, undiluted soil was used (100 mg kg^{-1} As, 1.0 M HCl extractable). The control soils (untreated field soils, pH(H_2O) = 6.0) contained 0.5 mg kg^{-1} Cd and 30 mg kg^{-1} As (0.1 M and 1.0 M HCl extractable), respectively. The soils used in this study originally contained relatively high concentrations of As due to the influence of the parent material.

Treatment and plant cultivation

In 2007, the pot experiment was conducted in a randomized complete block design with three replicates in a Hokkaido University greenhouse. Ten surface-sterilized Sunrise cultivar spinach seeds were sown on October 18th in 300-mL pots containing 300 g Cd- and As-contaminated soils and control soils (Cd, As, and Control, respectively) with (+Fe^0) and without (−Fe^0) 1.5 g Fe^0 (0.5 g per 100 g soil). The amount of Fe^0 added to the soil was determined based on our previous cultivation experiments using rice (Watanabe et al. 2009). Fifty mg N, 21.8 mg P (50 mg P_2O_5), and 41.5 mg K (50 mg K_2O) were added to each pot as ammonium sulfate, superphosphate, and potassium sulfate, respectively. The seedlings were thinned to five and grown in a greenhouse for 30 d. The soils were watered daily to maintain the water content at 55% of their maximum holding capacity (w/w). Fe^0 used in this study was an aggregated form (100-μm-diameter) of nanoscale iron particles, which was obtained from the JFE Steel Corporation (Tokyo, Japan).

Sampling and analysis

On November 17th, each plant at vegetative stage was harvested, separated into shoots and roots, and washed with deionized water. The samples were then dried in an oven at 70 °C for 7 d, weighed, and ground for mineral analysis. Cadmium and As concentrations in each organ were determined by inductively coupled plasma atomic emission spectrophotometry (ICP-AES; ICPS-7000, Shimadzu, Kyoto, Japan) after wet-digestion. Furthermore, the Cd- and As-contaminated air-dried soil samples were extracted with 0.1 and 1 M HCl (soil:HCl = 1:5, w/v), respectively, to determine the available Cd and As concentrations (Watanabe et al. 2015). Furthermore, the sequential extraction method (Sadamoto et al. 1994) was used to fractionate the Cd and As soils into five fractions. Briefly, soil samples (0.5 g) were shaken with 5 mL 0.05 M $Ca(NO_3)_2$ at 30 °C for 24 h and centrifuged at 3000 rpm for 10 min; the supernatant was defined as the exchangeable fraction. Next, the residue was shaken with 5 mL 2.5% CH_3COOH at 30 °C for 24 h and centrifuged at 3500 rpm for 15 min; the supernatant was defined as Fe-manganese (Mn)-oxide-bound fraction. Next, the residue was digested with H_2O_2 at 100 °C until the H_2O_2 evaporated, shaken with 5 mL 2.5% CH_3COOH at 30 °C for 24 h, and centrifuged at 3500 rpm for 20 min; the supernatant was defined as the organic-matter-bound fraction. Next, the residue was extracted with 15 mL acid ammonium oxalate (0.1 M oxalic acid, 0.236 M ascorbic acid, and 0.175 M ammonium oxalate) at 100 °C for 1 h and centrifuged at 15000 rpm for 10 min; the supernatant was defined as the free-oxide-occluded fraction. Next, the residue was vacuum-dried and ground in a mortar, and 100-mg samples were digested with HF-HCl-HNO_3; this fraction was defined as the residual fraction. The Cd and As concentrations in each fraction were determined using ICP-AES.

Statistical analyses

Means and standard errors of replicates were calculated using Excel 2013 (Microsoft, Redmond, WA, USA). Student's *t*-test was performed to determine the statistically significant differences between $-Fe^0$ and $+Fe^0$ treatments using Sigmaplot 14.5 (Systat Software, Inc., San Jose, CA, USA).

Results

Plant

There was no significant effect of Fe^0 application on the spinach dry weight after cultivation in the control and As treatments; however, it significantly decreased the dry weight in the Cd treatment (Fig. 1). Fe^0 significantly decreased Cd concentration in shoots and roots in the Cd treatment (Fig. 2), but the decrease in As concentration in the As treatment was not significant. However, Fe^0 significantly decreased As concentration in roots in the control (Fig. 2).

Soil

Fe^0 application had no significant effect on soil pH (Fig. 3). Fe^0 decreased the concentrations of 0.1 M HCl extractable Cd and 1 M HCl extractable As in the Cd and As treatment soils, respectively (Fig. 4). Additionally, Fe^0 significantly decreased the concentration of 1 M HCl extractable As in the control (Fig. 4). In the Cd treatment, Fe^0 significantly increased the Cd distribution in the free-oxide-occluded fraction ($P < 0.05$), while that in the organic-matter-bound and Fe-Mn-oxide-bound fractions decreased and increased, respectively ($P < 0.1$) (Fig. 5). In the As treatment, As mostly occurred in the free-oxide-occluded fraction, and its distribution increased with Fe^0 (Fig. 6); however, it was significantly reduced in the Fe-Mn-oxide-bound fraction, although its proportion to the total As was small. The proportion of As in the free-oxide-occluded fraction was also high in the control but lower than in the As treatment (Fig. S1). In control, as in the As treatment, Fe^0 increased the proportion of As in the free-oxide-occluded fraction and decreased it in the Fe-Mn-oxide-bound fraction (Fig. S1).

Discussion

In our previous study, rice growth was improved by applying Fe^0 to flooded Cd-contaminated soil (Watanabe et al. 2009). However, in this study, no beneficial effect of Fe^0 application was observed on spinach growth in the control, Cd-, or As-contaminated soils (Fig. 1). Contrarily, spinach growth in the Cd treatment was significantly reduced by Fe^0, possibly due to the different soil conditions, i.e., flooded (anaerobic) and non-flooded (aerobic) soils. However, it was unclear why growth was reduced in the Cd treatment only. Spinach is known to be sensitive to soil acidity (Abd El-Halim and Omae 2019). However, Fe^0 application did not significantly affect soil pH in all treatments (Fig. 3), suggesting that the growth reduction caused by Fe^0 in the Cd treatment was not due to soil acidity stress. Instead, this result may be due to the direct or indirect soil interactions between Cd and Fe^0; therefore, the negative effect of Fe^0 on growth needs further investigation.

Fe^0 application significantly reduced Cd concentration in both shoots and roots in the Cd treatment (Fig. 2). Fe^0 application also tended to decrease As concentration in the As treatment, but it was not statistically significant except in roots in the control treatment (Fig. 2). Since the field soil used in this study contained a relatively high As concentration (Fig. 4), it is likely that there was significant As accumulation even in the control treatment, which was suppressed by Fe^0 application. However, it should be noted that since the roots were only washed with deionized water, some of Cd or As deposited on the root surface may remain. Although a significant decrease in Cd concentration in the shoots indicates the effectiveness of Fe^0 in inhibiting Cd uptake, further study is necessary for As, as it exhibited no statistically significant effect on the shoots.

How then, did Fe^0 application affect Cd and As in the soil? The effect of Fe^0 on soil pH was negligible (Fig. 3), indicating pH variation was not the primary factor in Cd and As solubility. The 0.1 M HCl and 1 M HCl extraction methods are often used to estimate available Cd and As in soil (Matsumoto et al. 2015; Meers et al. 2007; Watanabe et al. 2015). The available Cd concentration in the Cd treatment soil and the available As concentration in the control and the As treatment soils determined by these extraction methods were significantly reduced by Fe^0 (Fig. 4). These results are consistent with the decrease of Cd and As (in roots of control) concentrations by Fe^0 application in spinach (Fig. 2).

The treatment soils were fractionated using the sequential extraction method (Sadamoto et al. (1994) to estimate the forms of Cd or As affected by Fe^0. The potential availability of Cd and As in the soil fractions is: exchangeable > Fe-Mn-oxide-bound > organic-matter-bound > free-oxide-occluded > residual. Fe^0 application significantly increased Cd distribution in the free-oxide-occluded fraction, and also tended to increase that in the Fe-Mn-oxide-bound fraction although not statistically significant (Fig. 5). Conversely, Cd distribution in the organic-matter-bound and exchangeable fractions tended to decrease. The change in Cd distribution in these fractions may have affected Cd accumulation in spinach. In a previous study using the same method to fractionate Cd-contaminated soil (same soil type as this study) under flooded conditions

(Watanabe et al. 2009), we found that Fe^0 application significantly increased Cd distribution in the free-oxide-bound fraction and decreased it in the exchangeable and Fe-Mn-oxide-bound fractions. These indicate that the effect of Fe^0 on soil Cd differs between flooded and non-flooded conditions. In this study, the degree of reduction in Cd accumulation in spinach due to Fe^0 was lower than in our previous study on rice (Fig. 2, Watanabe et al., 2009). Additionally, the difference in the effect of Fe^0 on the Cd forms, is possibly due to higher Cd availability under non-flooded (aerobic) conditions (higher percentage of exchangeable and Fe-Mn-oxide-bound) and lower reduction effect of Fe^0. The As distribution in the free-oxide-occluded fraction increased with Fe^0 application, while that in the Fe-Mn-oxide-bound fraction decreased (Fig. 6). In the control soil, As showed similar behavior (Fig. S1). These results suggest that the decrease in As accumulation in spinach roots due to Fe^0 soil application is due to a decrease in As in the Fe-Mn-oxide-bound fraction (relatively high availability) and an increase in the free-oxide-occluded fraction (relatively low available). The differences in the effects of Fe0 application on the chemical forms between Cd and As in the soil indicate that the mechanisms of Fe^0-induced immobilization of heavy metals and metalloids in soil are complicated, with multiple processes occurring simultaneously (Xue et al. 2018). Furthermore, since non-rhizosphere soils were used for the soil analysis in this study, the impact of plant roots, which may vary by plant species, has not been determined. Therefore, comprehensive analyses of the effects of Fe^0 on Cd and As in rhizosphere soils are needed to better understand their dynamics between plants and soils affected by Fe^0 application.

Conclusion

This study indicated that Fe^0 soil application could reduce Cd and As accumulation in spinach by changing the availabilities (forms) of Cd and As in soils. However, the effect of Fe^0 application was limited in this study because the degree of reduction in spinach Cd accumulation due to Fe^0 application was relatively low, and no significant reduction of As accumulation was observed in spinach shoots. In regard to this issue, it is expected that a higher application rate of Fe^0 will lead to increased effectiveness in reducing the accumulation of Cd and As in spinach. However, since Fe^0 materials are not inexpensive (Guan et al. 2015), for practical application of Fe^0 to Cd- and As-contaminated soils, it is essential to conduct comparative studies with other inexpensive materials to consider cost-effectiveness.

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Disclosure statement

The authors report no potential conflict of interest.

References

Abd El-Halim, A. A., and Omae, H. 2019. "Examination of nanoparticulate phosphate rock as both a liming agent and phosphorus source to enhance the growth of spinach in acid soil." *Soil Science and Plant Nutrition* 65 (4):386-92. doi: 10.1080/00380768.2019.1620082.

Abedi-Koupai, J., Mollaei, R., and Eslamian, S. S. 2015. "The effect of pumice on reduction of cadmium uptake by spinach irrigated with wastewater." *Ecohydrology & Hydrobiology* 15 (4):208-14. doi: https://doi.org/10.1016/j.ecohyd.2015.05.001.

Alexander, P. D., Alloway, B. J., and Dourado, A. M. 2006. "Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables." *Environmental Pollution* 144 (3):736-45. doi: https://doi.org/10.1016/j.envpol.2006.03.001.

Alkorta, I., Hernandez-Allica, J., Becerril, J. M., Amezaga, I., Albizu, I., and Garbisu, C. 2004. "Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic." *Reviews in Environmental Science and Biotechnology* 3:71-90. doi: 10.1023/B:RESB.0000040059.70899.3d.

Arao, T., Ishikawa, S., Murakami, M., Abe, K., Maejima, Y., and Makino, T. 2010. "Heavy metal contamination of agricultural soil and countermeasures in Japan." *Paddy and Water Environment* 8 (3):247-57. doi: 10.1007/s10333-010-0205-7.

Bergqvist, C., Herbert, R., Persson, I., and Greger, M. 2014. "Plants influence on arsenic availability and speciation in the rhizosphere, roots and shoots of three different vegetables." *Environmental Pollution* 184:540-6. doi: https://doi.org/10.1016/j.envpol.2013.10.003.

Bramley, R. G. V. 1990. "Cadmium in New Zealand agriculture." *New Zealand Journal of Agricultural Research* 33 (4):505-19. doi: 10.1080/00288233.1990.10428451.

Clemens, S., and Ma, J. F. 2016. "Toxic Heavy Metal and Metalloid Accumulation in Crop Plants and Foods." *Annual Review of Plant Biology* 67 (1):489-512. doi: doi:10.1146/annurev-arplant-043015-112301.

Dheri, G. S., Singh Brar, M., and Malhi, S. S. 2007. "Influence of phosphorus application on growth and cadmium uptake of spinach in two cadmium-contaminated soils." *Journal of Plant Nutrition and Soil Science* 170 (4):495-9. doi: https://doi.org/10.1002/jpln.200625051.

Guan, X., Sun, Y., Qin, H., Li, J., Lo, I. M. C., He, D., and Dong, H. 2015. "The limitations of applying zero-valent iron technology in contaminants sequestration and the corresponding countermeasures: The development in zero-valent iron technology in the last two decades (1994–2014)." *Water Research* 75:224-48. doi:

https://doi.org/10.1016/j.watres.2015.02.034.

Hanauer, T., Felix-Henningsen, P., Steffens, D., Kalandadze, B., Navrozashvili, L., and Urushadze, T. 2011. "In situ stabilization of metals (Cu, Cd, and Zn) in contaminated soils in the region of Bolnisi, Georgia." *Plant and Soil* 341 (1):193-208. doi: 10.1007/s11104-010-0634-5.

Hashimoto, Y., and Yamaguchi, N. 2013. "Chemical speciation of cadmium and sulfur K-edge XANES spectroscopy in flooded paddy soils amended with zerovalent iron." *Soil Science Society of America Journal* 77 (4):1189-98. doi: https://doi.org/10.2136/sssaj2013.01.0038.

Huang, Y., He, C., Shen, C., Guo, J., Mubeen, S., Yuan, J., and Yang, Z. 2017. "Toxicity of cadmium and its health risks from leafy vegetable consumption." *Food & Function* 8 (4):1373-401. doi: 10.1039/C6FO01580H.

John, M. K. 1973. "Cadmium uptake by eight food crops as influenced by various soil levels of cadmium." *Environmental Pollution (1970)* 4 (1):7-15. doi: https://doi.org/10.1016/0013-9327(73)90026-8.

Lien, H.-L., and Wilkin, R. T. 2005. "High-level arsenite removal from groundwater by zero-valent iron." *Chemosphere* 59 (3):377-86. doi: 10.1016/j.chemosphere.2004.10.055.

Liu, L., Li, W., Song, W., and Guo, M. 2018. "Remediation techniques for heavy metal-contaminated soils: Principles and applicability." *Science of The Total Environment* 633:206-19. doi: https://doi.org/10.1016/j.scitotenv.2018.03.161.

Matsumoto, S., Kasuga, J., Taiki, N., Makino, T., and Arao, T. 2015. "Inhibition of arsenic accumulation in Japanese rice by the application of iron and silicate materials." *CATENA* 135:328-35. doi: https://doi.org/10.1016/j.catena.2015.07.004.

Meers, E., Du Laing, G., Unamuno, V., Ruttens, A., Vangronsveld, J., Tack, F. M. G., and Verloo, M. G. 2007. "Comparison of cadmium extractability from soils by commonly used single extraction protocols." *Geoderma* 141 (3):247-59. doi: https://doi.org/10.1016/j.geoderma.2007.06.002.

Mlangeni, A. T., Perez, M., Raab, A., Krupp, E. M., Norton, G. J., and Feldmann, J. 2020. "Simultaneous stimulation of arsenic methylation and inhibition of cadmium bioaccumulation in rice grain using zero valent iron and alternate wetting and drying water management." *Science of The Total Environment* 711:134696. doi: https://doi.org/10.1016/j.scitotenv.2019.134696.

Rahaman, S., Sinha, A. C., Pati, R., and Mukhopadhyay, D. 2013. "Arsenic contamination: a potential hazard to the affected areas of West Bengal, India." *Environmental Geochemistry and Health* 35 (1):119-32. doi: 10.1007/s10653-012-9460-4.

Rangsivek, R., and Jekel, M. R. 2005. "Removal of dissolved metals by zero-valent iron (ZVI): Kinetics, equilibria, processes and implications for stormwater runoff treatment." *Water Research* 39 (17):4153-63. doi: 10.1016/j.watres.2005.07.040.

Roberts, J. L., and Moreau, R. 2016. "Functional properties of spinach (Spinacia oleracea L.) phytochemicals and bioactives." *Food & Function* 7 (8):3337-53. doi: 10.1039/C6FO00051G.

Sadamoto, H., K, I., T, H., and S, Y. 1994. "Examination of fractionation of heavy metals in soils." *Japanese Journal of Soil Science and Plant Nutrition* 65:645-53. doi: 10.20710/dojo.65.6_645.

Sato, A., Takeda, H., Oyanagi, W., Nishihara, E., and Murakami, M. 2010. "Reduction of cadmium uptake in spinach (Spinacia oleracea L.) by soil amendment with animal waste compost." *Journal of Hazardous Materials* 181 (1):298-304. doi: https://doi.org/10.1016/j.jhazmat.2010.05.011.

Upadhyay, M. K., Shukla, A., Yadav, P., and Srivastava, S. 2019. "A review of arsenic in crops, vegetables, animals and food products." *Food Chemistry* 276:608-18. doi: https://doi.org/10.1016/j.foodchem.2018.10.069.

Watanabe, T., Maejima, E., Yoshimura, T., Urayama, M., Yamauchi, A., Owadano, M., Okada, R., Osaki, M., Kanayama, Y., and Shinano, T. 2016. "The Ionomic Study of Vegetable Crops." *PLoS ONE* 11 (8):e0160273. doi: 10.1371/journal.pone.0160273.

Watanabe, T., Murata, Y., Nakamura, T., Sakai, Y., and Osaki, M. 2009. "Effect of zero-valent iron application on cadmium uptake in rice plants grown in cadmium-contaminated soils." *Journal of Plant Nutrition* 32 (7):1164-72. doi: 10.1080/01904160902943189.

Watanabe, T., Urayama, M., Shinano, T., Okada, R., and Osaki, M. 2015. "Application of ionomics to plant and soil in fields under long-term fertilizer trials." *SpringerPlus* 4 (1):1-13. doi: 10.1186/s40064-015-1562-x.

Xue, W., Huang, D., Zeng, G., Wan, J., Cheng, M., Zhang, C., Hu, C., and Li, J. 2018. "Performance and toxicity assessment of nanoscale zero valent iron particles in the remediation of contaminated soil: A review." *Chemosphere* 210:1145-56. doi: https://doi.org/10.1016/j.chemosphere.2018.07.118.

Figure captions

Figure 1. Dry weight of spinach plants grown in soils with different treatments.

Values are means of three replicates. Range indicates ± standard error for total dry weight.

Asterisks indicate significant differences in total dry weight between without ($-Fe^0$) and with ($+Fe^0$) zero-valent iron applications in each treatment (Student's t-test, **: $P < 0.01$). Cd: cadmium; As: arsenic.

Figure 2. Cadmium and arsenic concentration in spinach shoots and roots in soils with different treatments

Values are means of three replicates. Range indicates ±standard error. Asterisks indicate significant differences in concentration between without ($-Fe^0$) and with ($+Fe^0$) zero-valent iron applications in each treatment (Student's t-test, * and ***: $P < 0.05$ and 0.001, respectively). Cd: cadmium; As: arsenic.

Figure 3. Effect of zero-valent iron application on soil pH in each treatment.

Values are the means of three replicates. Range indicates ± standard error. No significant difference was observed between without ($-Fe^0$) and with ($+Fe^0$) zero-valent iron applications in each treatment (Student's t-test, $P <0.05$). Cd: cadmium; As: arsenic.

Figure 4. Effect of zero-valent iron application on 0.1 M HCl extractable cadmium and 1 M HCl extractable arsenic in soil.

Values are the means of three replicates. Range indicates ± standard error. Asterisks indicate significant differences in concentration between without ($-Fe^0$) and with ($+Fe^0$) zero-valent iron applications in each treatment (Student's t-test, * and **: $P < 0.05$ and 0.01, respectively). Cd: cadmium; As: arsenic.

Figure 5. Effect of zero-valent iron application on the distribution of cadmium in each

fraction of Cd treatment soil.

Asterisks indicate statistically significant differences in distribution ratio between without (−Fe^0) and with (+Fe^0) zero-valent iron applications (Student's t-test, **: $P < 0.01$). For reference, not statistically significant ($P > 0.05$) but showing a trend ($P < 0.1$) is indicated with †. Cd: cadmium.

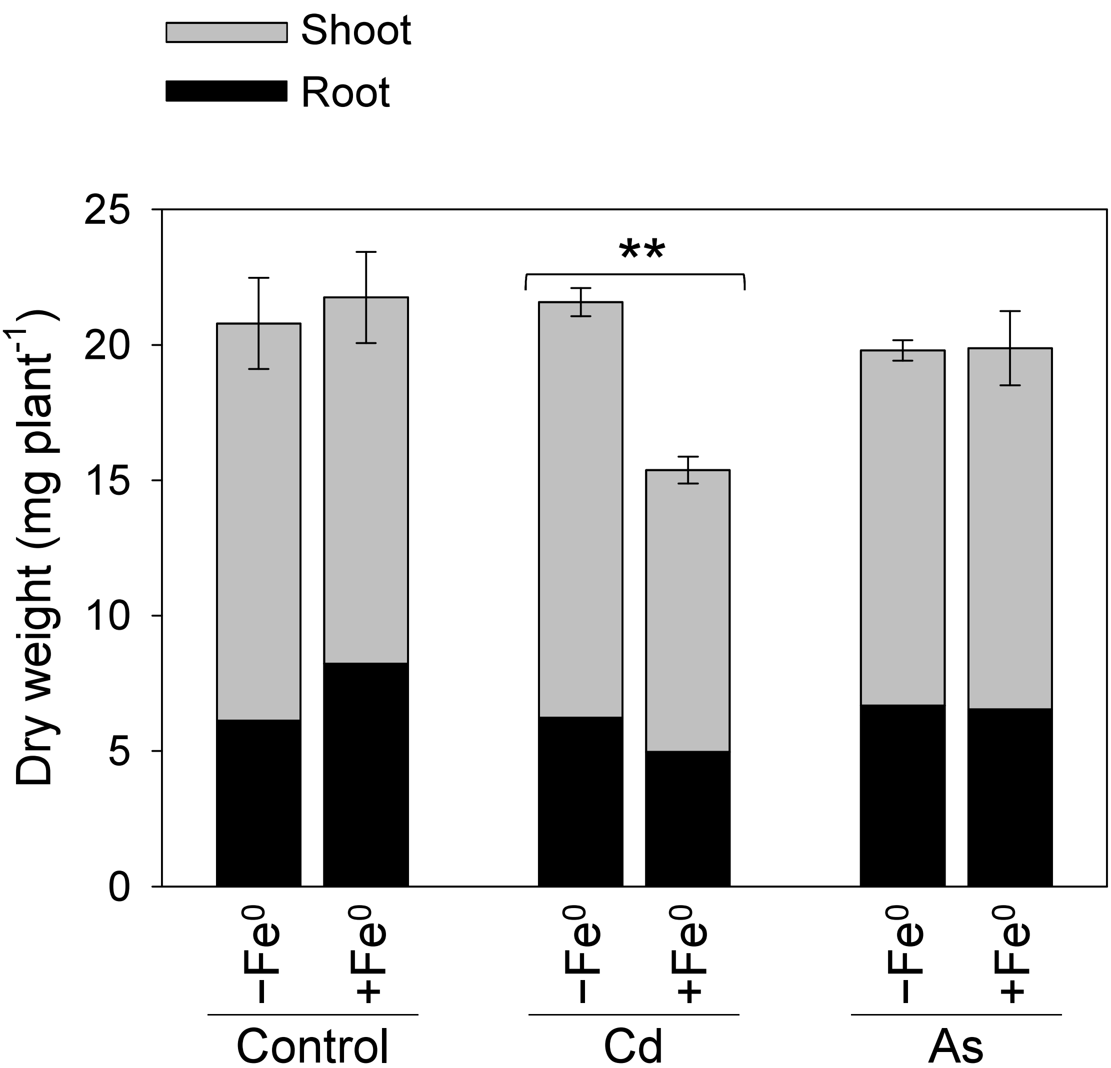
Figure 6. Effect of zero-valent iron application on the distribution of arsenic in each fraction of As treatment soil.

Asterisks indicate significant differences in distribution ratio between without (−Fe^0) and with (+Fe^0) zero-valent iron applications (Student's t-test, **: $P < 0.01$). Graph below is an enlargement of a portion of horizontal axis of graph above. As: arsenic.

Supplemental data

Figure S1. Effect of zero-valent iron application on the distribution of arsenic in each fraction of As treatment soil.

Asterisks indicate significant differences in distribution ratio between without (−Fe^0) and with (+Fe^0) zero-valent iron applications (Student's t-test, *: $P < 0.05$). For reference, † indicates no significant difference ($P > 0.05$) but showing a trend ($P < 0.1$). As: arsenic.



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Values are means of three replicates. Range indicates ± standard error for total dry weight. Asterisks indicate significant differences in total dry weight between without ($-Fe^0$) and with ($+Fe^0$) zero-valent iron applications in each treatment (Student's t-test, **: $P < 0.01$). Cd: cadmium; As: arsenic.

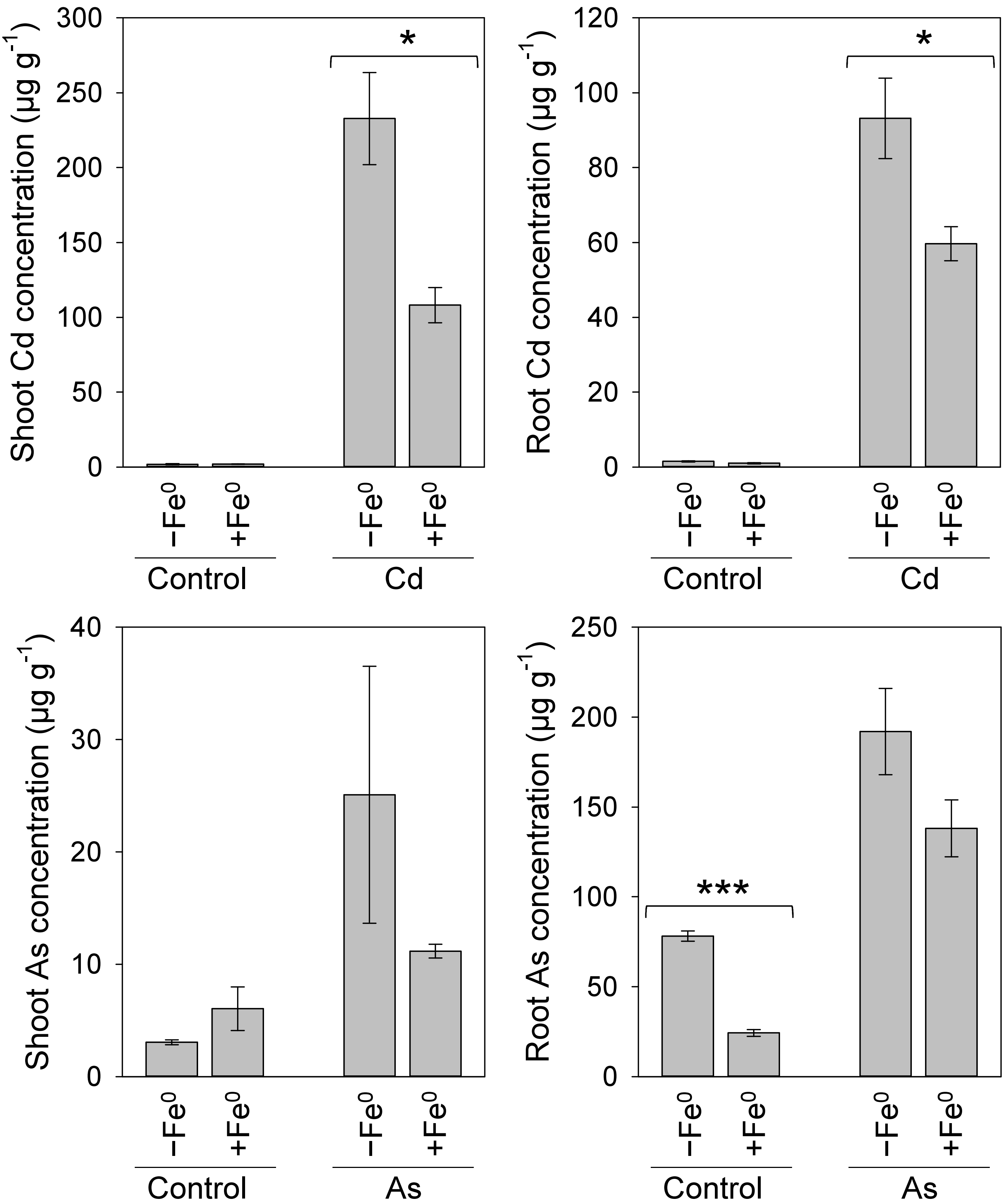

Figure 2. Cadmium and arsenic concentration in spinach shoots and roots in soils with different treatments

Values are means of three replicates. Range indicates ±standard error. Asterisks indicate significant differences in concentration between without ($-Fe^0$) and with ($+Fe^0$) zero-valent iron applications in each treatment (Student's t-test, * and ***: $P < 0.05$ and 0.001, respectively). Cd: cadmium; As: arsenic.

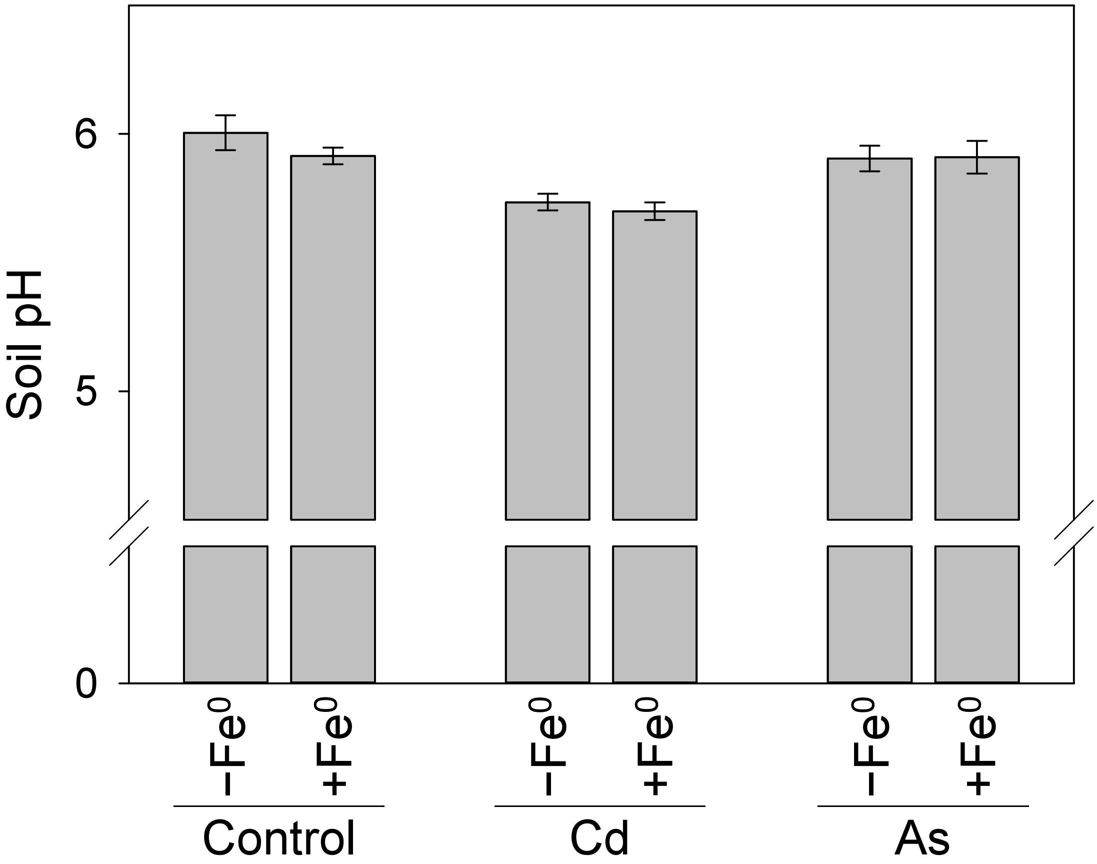

Figure 3. Effect of zero-valent iron application on soil pH in each treatment.
Values are the means of three replicates. Range indicates ± standard error. No significant difference was observed between without ($-Fe^0$) and with ($+Fe^0$) zero-valent iron applications in each treatment (Student's t-test, $P < 0.05$). Cd: cadmium; As: arsenic.

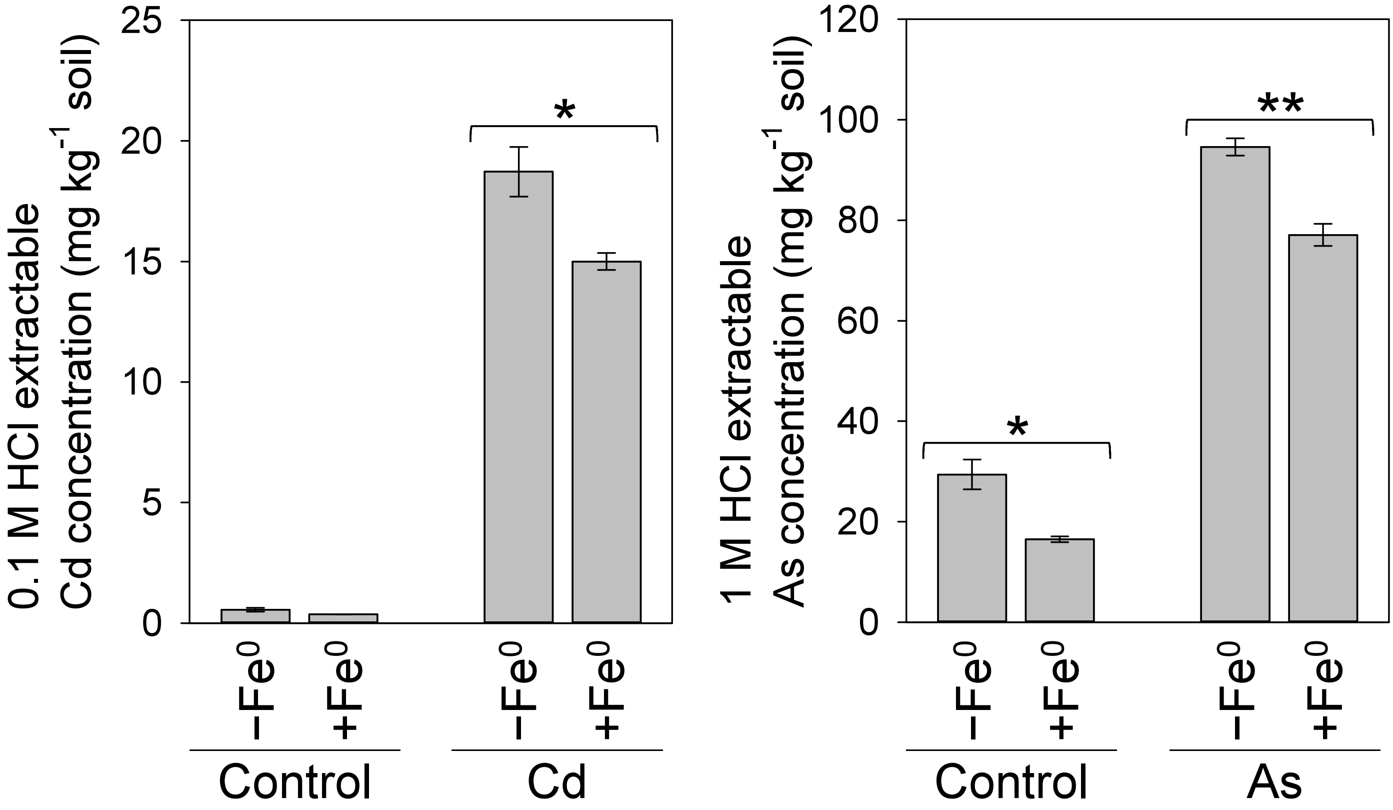

Figure 4. Effect of zero-valent iron application on 0.1 M HCl extractable cadmium and 1 M HCl extractable arsenic in soil.

Values are the means of three replicates. Range indicates ± standard error. Asterisks indicate significant differences in concentration between without (−Fe^0) and with (+Fe^0) zero-valent iron applications in each treatment (Student's t-test, * and **: $P < 0.05$ and 0.01, respectively). Cd: cadmium; As: arsenic.

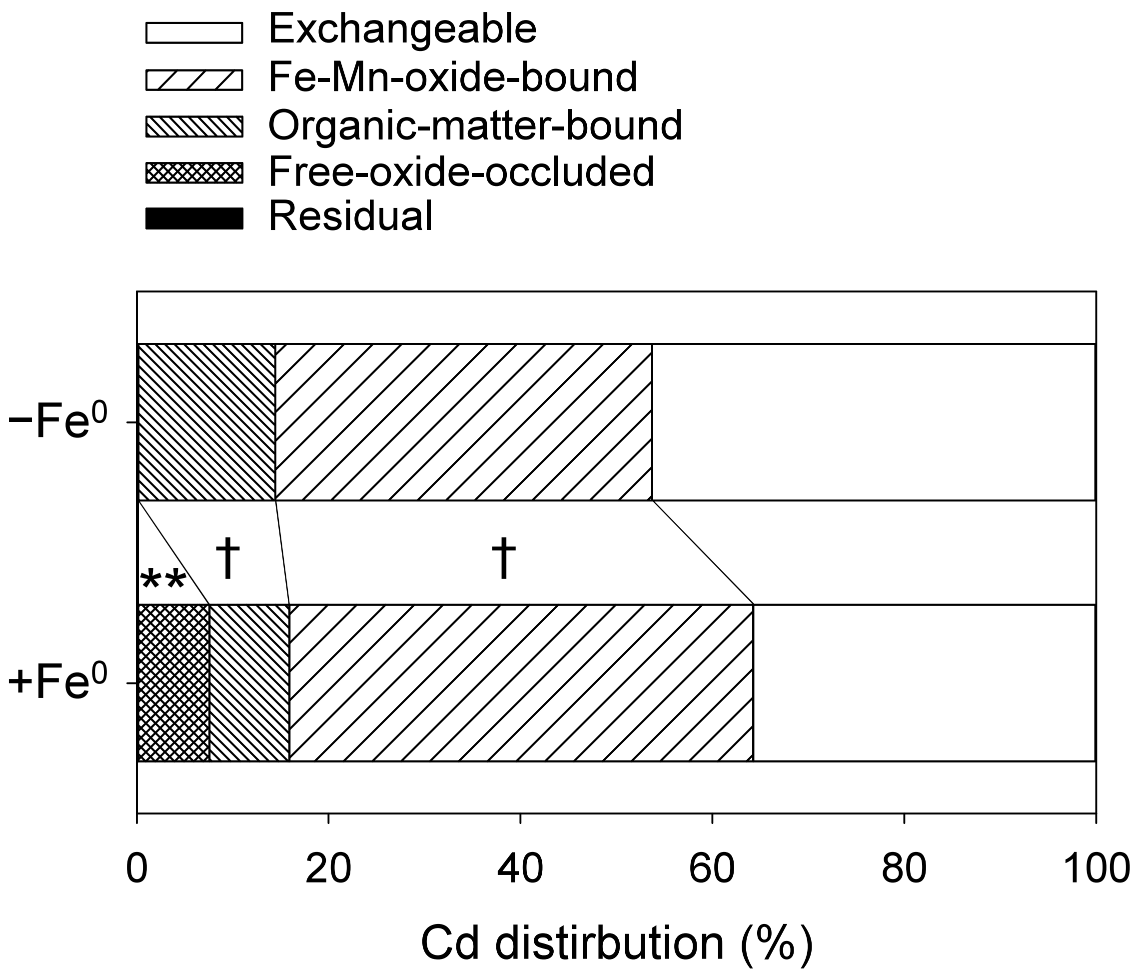

Figure 5. Effect of zero-valent iron application on the distribution of cadmium in each fraction of Cd treatment soil.

Asterisks indicate statistically significant differences in distribution ratio between without ($-Fe^0$) and with ($+Fe^0$) zero-valent iron applications (Student's t-test, **: $P < 0.01$). For reference, not statistically significant ($P > 0.05$) but showing a trend ($P < 0.1$) is indicated with †. Cd: cadmium.

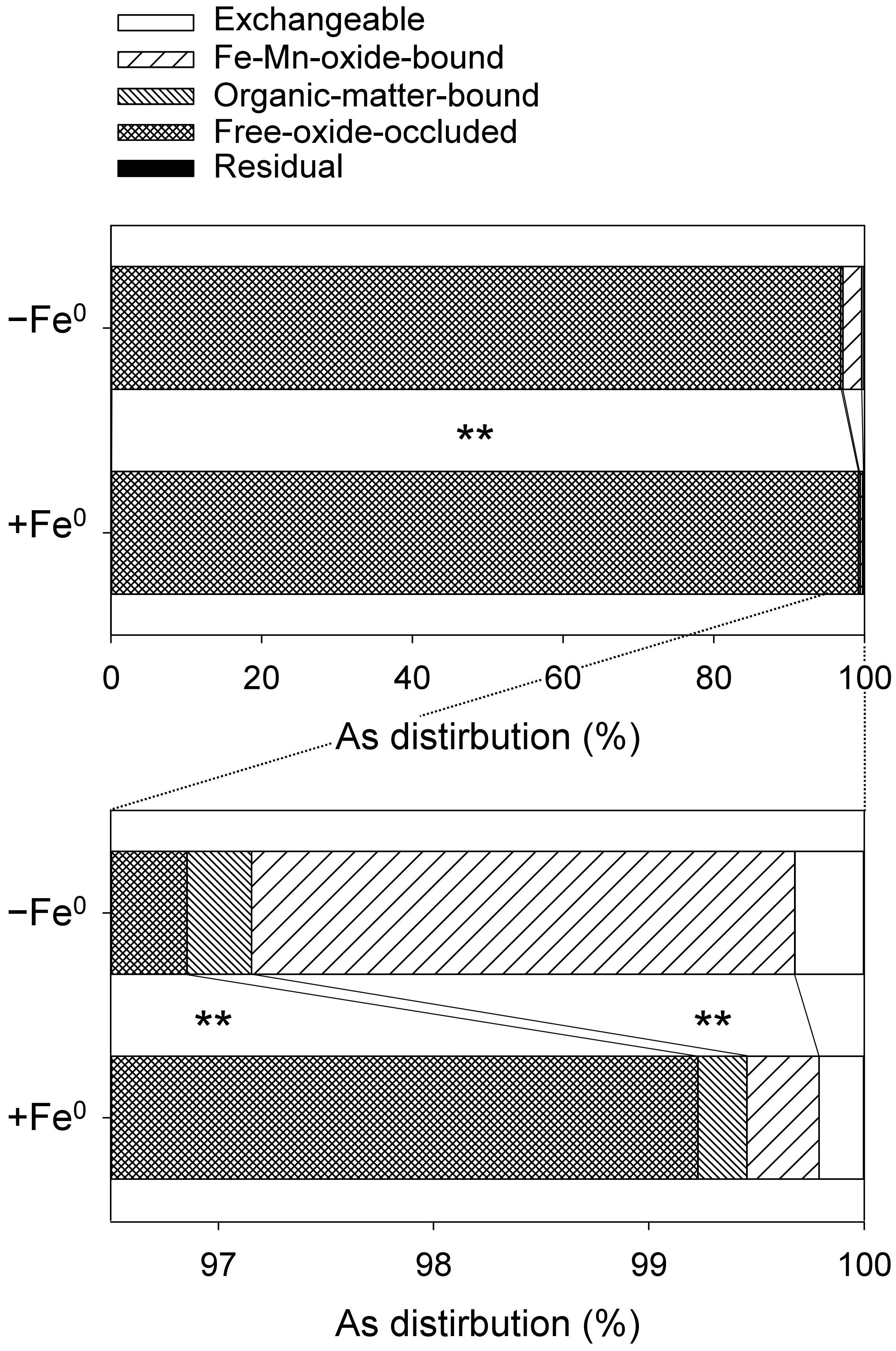

Figure 6. Effect of zero-valent iron application on the distribution of arsenic in each fraction of As treatment soil.

Asterisks indicate significant differences in distribution ratio between without ($-Fe^0$) and with ($+Fe^0$) zero-valent iron applications (Student's t-test, **: $P < 0.01$). Graph below is an enlargement of a portion of horizontal axis of graph above. As: arsenic.