

# Supplementary Materials

## Remediation of Cd-Contaminated Soil by Modified Nanoscale Zero-Valent Iron: Role of Plant Root Exudates and Inner Mechanisms

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### Text S1 Nanoparticles Synthesis and Characterization

The synthetic procedure was adapted from the experiments of Huang and Xue et al. [1-3]. Briefly, the nZVI and CMC-nZVI were synthesized through the reduction of ferrous sulfate by sodium borohydride at the  $\text{BH}_4^-/\text{Fe}^{2+}$  molar ratio of 2.0. And the entire process was carried out in solution under nitrogen atmosphere with mechanical stirring. The only difference was the absence or addition of various concentrations of CMC (0, 0.1, 0.15, 0.2, 0.25 wt%) to the solution. After synthesis, the nanoparticles were separated by magnetic force and then washed several times with deoxygenated deionized water (18.25M $\Omega$  cm, Barnstead D11911) and alcohol to remove impurities before being dried in a vacuum oven and stored in brown bottles filled with nitrogen.

Scanning Electron Microscope (SEM) (Zeiss Gemini 300) was used to obtain the SEM images of nZVI and CMC-nZVI for observing their superficial morphological characteristics. X-ray diffraction (XRD) patterns of nZVI and CMC-nZVI were determined to analyze the mineralogy by a powder XRD system (Bruker, AXS D8 Advance). The diffraction angle is scanned from 5° to 90° at a rate of 2°/min.

### Text S2 Fractions of Cd in the soil samples

The speciations of Cd in samples were measured by the modified sequential extraction method of European Community Bureau of Reference (BCR) [4,5]. The extractions were divided into four steps corresponding to four fractions separately. Acid extractable/exchangeable fraction (i.e. carbonates and the exchangeable fraction): for step 1, 40 mL of 0.11 M acetic acid was added to a centrifuge tube containing 0.5 g of sample and shaken for 16 h at room temperature. Subsequently, the supernatant was removed by

centrifugation for detection, while the solid residue was washed by centrifugation with deionized water which was then decanted for step 2. Reducible fraction (i.e. iron and manganese oxides fraction): 40 mL of freshly prepared hydroxylammonium chloride was added to the residue of step 1. The following steps were the same as above. Oxidizable fraction (i.e. organic and sulfide fraction): the residue from step 2 was treated by 10 mL of 8.8 M hydrogen peroxide twice. Digestion was carried out for 1 h at room temperature with occasional manual shaking, followed by an additional digestion for 1 h in a water bath at  $85 \pm 2^\circ\text{C}$  until the mixture volume reduced to 2-3 mL. The previous step was repeated again and 50 mL of 1.0 M ammonium acetate was added to the residue in the tube, followed by 16 h of shaking at room temperature. Residual fraction (i.e. the metals in the crystalline lattice of primary and secondary minerals): the residue of step 3 was digested with aqua regia and hydrofluoric acid. All the above extracts were filtered through a  $0.45 \mu\text{m}$  organic filter membrane and then measured by atomic absorption spectrometry.

### **Text S3 Methods for the detection of soil physico-chemical properties**

Soil pH was measured by diffusing 1 g of soil into water at a ratio of 1:5 w/w of soil to water and determined through inserting the electrode of a pH meter into the supernatant.

The method for the determination of soil OM content was obtained from the previous research, which was known as the potassium dichromate volumetric method-the dilution heating method [6]. The principle is to oxidize soil organic matter with a known concentration of potassium dichromate-sulfuric acid solution under external heating conditions. The OM value was calculated by the following equation:

$$\text{OM}(\text{g/kg}) = \frac{0.5 \times (V_0 - V) \times 0.001 \times 3.0 \times 1.33}{M} \times 1000 \times 1.724 \quad (1)$$

where  $V_0$  is the  $\text{FeSO}_4$  volume consumed by silicon dioxide of the blank, the  $V$  is the consumption of the  $\text{FeSO}_4$  by the sample and the  $M$  is the weight of the soil sample.

The CEC value was determined by barium chloride - sulfuric acid enhanced exchange method [4]. The principle is that the various cations present in the soil can be equivalently exchanged with the  $\text{Ba}^{2+}$  in the barium chloride ( $\text{BaCl}_2$ ) solution. The  $\text{Ba}^{2+}$  exchanged into the soil are then exchanged out by strong electrolyte sulfuric acid ( $\text{H}_2\text{SO}_4$ ) solution, and due to the generation of barium sulfate ( $\text{Ba}_2\text{SO}_4$ ) precipitate, and the exchange and adsorption capacity of hydrogen ions is very strong, so that the exchange tends to be complete. Thus, by measuring the change of sulfuric acid content before and after the exchange reaction, the result of the CEC can be calculated using the equation as follows:

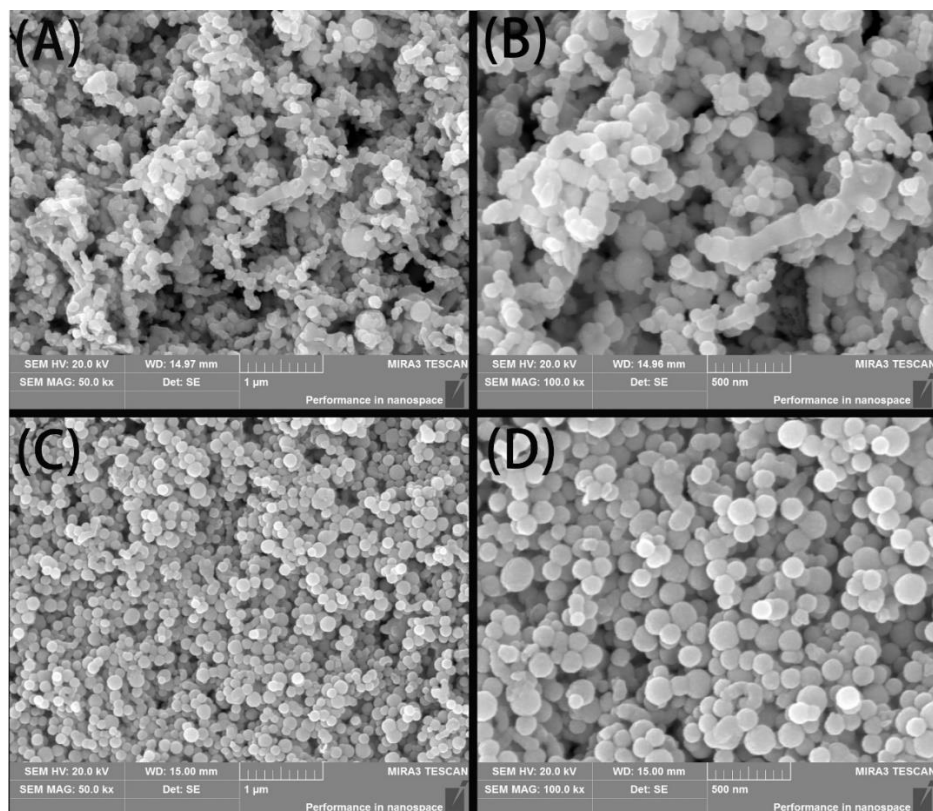
$$\text{CEC} \left( \frac{\text{cmol}}{\text{kg}} \right) = \frac{[(C_H \times 50) - (C_N \times V_N)] \times 100}{W_0 K_2} \quad (2)$$

where  $C_H$  is the concentration of  $\text{H}_2\text{SO}_4$  solution,  $C_N$  is the concentration of  $\text{NaOH}$  solution,  $V_N$  is the volume consumption of

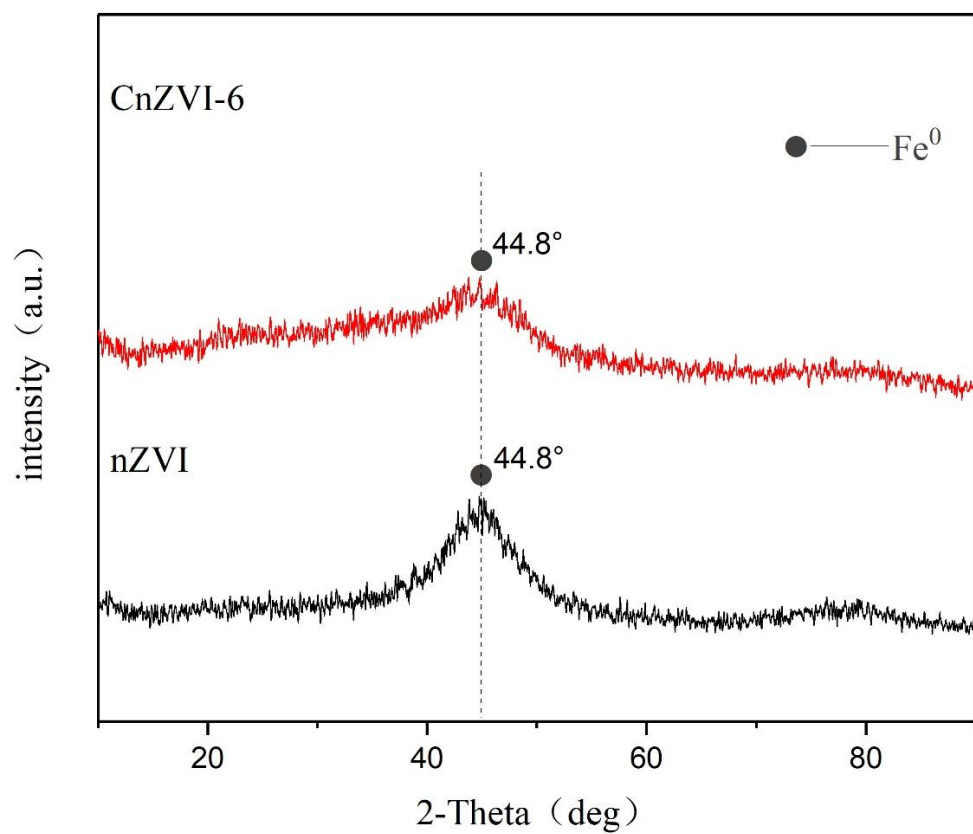
NaOH solution,  $W_0$  is the weight of the soil sample and  $K_2$  represent the moisture content of the sample.

#### **Text S4 High-throughput sequencing**

The primers for amplification of bacterial 16S rRNA in the V4 region used in this experiment were 338 F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806 R (5'-GGACTACNVGGGTWTCTAA-3') [7]. In this study, high-throughput sequencing was performed on the PCR products of specific fragments such as 16S rRNA functional genes to explore the evolution of soil microbial community after the above treatment, followed by investigating the inner mechanisms of simulated rhizosphere environment. Five samples, (Z0 (control group), nZVI\_0 (nZVI only), CnZVI\_0 (CnZVI only), CnZVI\_1 (CnZVI + 50mg/L CA), and CnZVI\_10 (CnZVI +500 mg/L CA)), were preincubated for 30 days and then outsourced to Shanghai Majorbio Bio-pharm Technology Co., Ltd, China for the high-throughput sequencing. The final data were obtained from the free online platform of Majorbio Cloud Platform ([www.majorbio.com](http://www.majorbio.com)).



**Figure S1.** SEM images of bare nZVI under (A) 1  $\mu\text{m}$  scale and (B) 500 nm scale, and CnZVI-6 under (C) 1  $\mu\text{m}$  scale and (D) 500 nm scale.



**Figure S2.** XRD spectra of freshly prepared bare nZVI and CnZVI-6.

## Reference

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