

Original Research

Palygorskite Loaded with Nanoscale Zero-Valent Iron as an Effective Stabilizer for Remediation of Soil Contaminated by Cd and Pb

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Abstract

Nowadays, the remediation of agricultural soils polluted by Cd and Pb is urgently required. In this study, we used the palygorskite (PAL) as a carrier for nanoscale zero-valent iron (nZVI) to synthesize a new type of stabilizer (PAL-nZVI). After 45 days of incubation, the soil pH increased by 1.80 pH units. The batch experiments exhibited that the bioavailability and leaching toxicity decreased significantly ($P < 0.05$) with the addition of PAL-nZVI. The data concluded that the 30 days was a critical period for Cd and Pb stabilization and the optimum dosage of PAL-nZVI was 6%. Moreover, the application of PAL-nZVI could transform the speciation of Cd and Pb from labile fraction to stable fraction (the maximum residual percentage of Cd and Pb increased by 27.34% and 36.57%, respectively) after 30 days of incubation. The pot experiments indicated that the application of PAL-nZVI could enhance the growth of corn (*Zea mays* L.), reduce the accumulation of heavy metals from the soil and decrease the phytotoxicity of Cd and Pb in the soil. These results suggested that the PAL-nZVI, which is high-efficiency and low-cost, can be a feasible stabilizer for the soils contaminated by Cd and Pb.

Keywords: Palygorskite, nanoscale zero-valent iron, stabilization, heavy metals, soil

Introduction

Environmental problems in agricultural soils with increasing heavy metal pollutants have become a worldwide issue over the last few decades, which is crucial for the environmental safety and human health [1-3]. Heavy metal accumulation in the soil is a

complex and dynamic process which affected by many factors, such as mining, smelting, traffic emissions and other anthropogenic activities [4]. Cadmium (Cd) and lead (Pb) are regarded as the primary poisonous elements which are widespread in soil due to their higher toxicity and bioaccumulation than other heavy metals [5-7]. According to the first national report by the Chinese government, 19.4% of Chinese agricultural soils had been contaminated by heavy metals, and the over-standard rate of Cd and Pb attained 7% and 1.5%, respectively. Because of the high bioaccumulation of

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heavy metal, they would hardly be degraded in the ecosystem and permanently exist when they enter the soil and groundwater. This non-biodegradability causes another hot issue need to be vigilant in China-food safety. When the heavy metal accumulated in human and animals through the soil-crop food chain, it could cause severe impairment for immune, nervous and endocrine systems and destroy the function of the liver, fertility and kidneys [8]. Therefore, it is imperative to develop a practicable and effective method to reduce the toxicity of heavy metals and remediate the Cd and Pb-contaminated soil.

Currently, there are a large number of techniques have been employed to remediate the soil contaminated by heavy metals, such as electrokinetic remediation, coagulation, phytoremediation, in-situ stabilization and leaching technology [9]. Among these techniques, *in-situ* stabilization, which is not limited by the cost and application scale, has been getting great attention around the world. In order to choose a cheap, eco-friendly and readily available material as the in-situ adsorbent, many materials have been tested, containing zeolite [10-11], lime [12], biochar [13], sepiolite [14] and hydroxyapatite [15]. Nanoscale zero-valent iron (nZVI), relies on its particular core-shell construction, superior reactivity and high adsorption capacity, which has become a potential remediation material [16-17]. It has attracted much attention to improving the quality of heavy metal polluted soil [18-19]. Unfortunately, there are still obvious technical challenges that hinder its application in the field. Because of the magnetic, van der Waals and its nano-size effects, it could be oxidized and aggregated immediately, which reduces the reaction activity sites and then decrease the sorption capacity [20-21]. To address this deficiency, the incorporation of another supported material is one of the feasible strategies to overcome these troubles of nZVI [22-23].

Palygorskite, a kind of natural inorganic mineral material, possesses a large number of active sites on the internal and external surface due to its mesoporous structures, which could be used as an effective amendment to remediate the combined contaminated soil [24-28]. Hence, the palygorskite should be a suitable supported material for nZVI to enhance its active sites and improve its sorption capacity. On the one hand, the palygorskite can serve as a considerable carrier because of its large specific surface and high thermal stability. When the nanoscale zero-valent iron coated with palygorskite, it could disperse uniformly on the surface of palygorskite as the nano-particles to prevent its aggregation. On the other hand, because a large number of useful functional groups (-OH, \equiv Si-OH, etc.), which exist on the surface of palygorskite, could stabilize the heavy metals by complexation, cation exchange or electrostatic action [29-30]. The possible stabilization mechanism of Cd and Pb by PAL-nZVI was shown in Fig. 1. What's more, nanoscale zero-valent iron is a relatively expensive material, the cost of remediation can be decreased significantly when the palygorskite

load with nanoscale zero-valent iron [31]. However, very little reports use the palygorskite modified with nZVI to remediate the heavy metals contaminated soil. Most of them concentrated on applying the amendment in the aqueous solutions [31-33], and the stabilization mechanisms of PAL-nZVI and ecological risks in the soil is unclear.

The main objectives of this study were to (1) synthesize and characterize the PAL-nZVI; (2) elucidate the change of amended-soil property; (3) investigate the optimum dosage and reaction time for immobilization efficiency of Cd and Pb in the soil; (4) explore the change of Cd and Pb speciation in the soil after amended by PAL-nZVI and explain the stabilization mechanisms; (5) confirm whether PAL-nZVI could decrease the phytotoxicity of Cd and Pb in polluted soil and promote the growth of the plant.

Material and Methods

Soil Description

The metal-contaminated soil samples with 19.15 ± 0.58 mg/kg Cd and 94.21 ± 1.46 mg/kg Pb was collected from the surface layer (0-20 cm) in Baiyin, Gansu, Northwest China ($36^{\circ}29'39''$ N, $104^{\circ}16'5''$ E). The soil was contaminated with cadmium and lead severely because of the historical lead-zinc smelting and mining. It was a low-permeability clay layer that tended to accumulate heavy metals in the discharged industrial effluents [34]. Now the land is still used as cropland and the plants, such as corns, celeries are planted in the soil. The soil samples were air-dried, ground and sieved with a 2-mm mesh, then the samples were stored in the dryer for future use. The concentration of Cd and Pb in soil were determined by atomic absorption spectrometry (AAS, Persee TAS-990) after digesting with HNO_3 -HCl- HClO_4 (v:v:v = 1:3:1). The other essential physicochemical properties of soil were pH of 4.82 ± 0.02 , cation exchange capacity of 12.8 ± 1.5 cmol/kg; EC of 2.11 ± 0.04 dS/m; organic matter of $2.94 \pm 0.3\%$.

Preparation and Characterization of Materials

Palygorskite was purchased from Gansu Hanxin Environmental Protection Co. Ltd. and collected from Banqiao town, Linze county of Gansu province, China. The ingredients were palygorskite of 36%, quartz of 28%, feldspar of 11%, dolomite of 15%, chlorite of 7% and muscovite of 1-2%, respectively. Before use, the palygorskite was acid-activated by the 4 M HCl for 24 h at room temperature. Centrifugation to separate the solid from solution and washed the PAL until neutral (pH = 7 ± 0.5) with deionized water. The acid-activated PAL was dried at 105°C , and then calcinated in a muffle furnace for 2 h at 400°C .

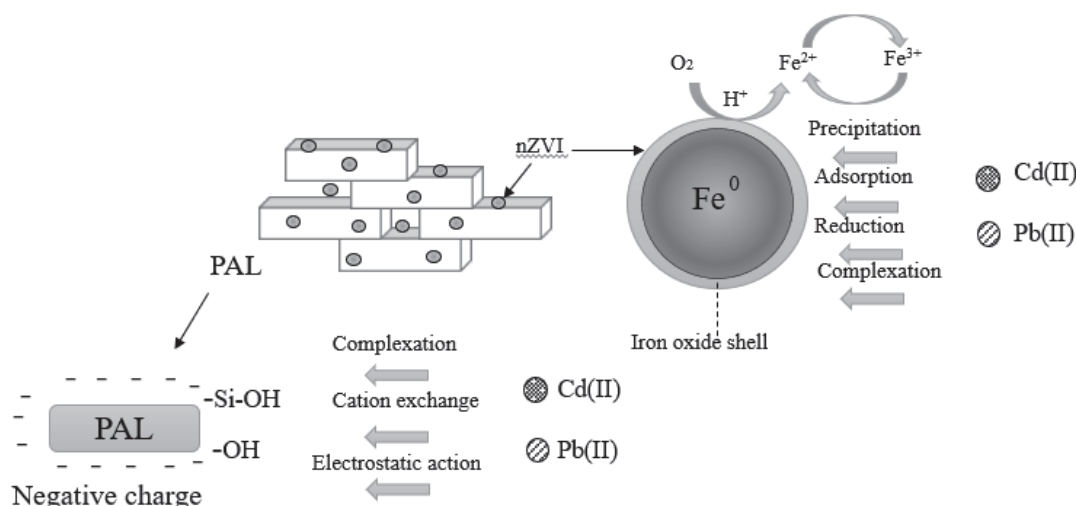


Fig. 1. The possible stabilization mechanism of Cd and Pb by PAL-nZVI [29-30].

Then the sample was sieved through a 0.149 mm mesh to use.

The PAL-nZVI with a palygorskite/iron mass ratio of 2:1 was synthesized by the following method: 20 g PAL was added to 500 mL 0.18 mol/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (99.5%) and stirred 12 h at $25 \pm 2^\circ\text{C}$ using an electrical stirrer. Secondly, the mixture was transferred to a three-necked flask, 500 mL of freshly prepared KHB_4 (0.8 mol/L) solution was added dropwise into the mixture while constantly stirring for another 1 h, and the entire process was under the protection of high purity nitrogen. Then, separating the black products from the liquid phase and washed solids with deoxygenated ultrapure water and deoxygenated absolute ethanol for several times to remove the residual chloride and borohydride. Finally, put the products in a vacuum drying oven at 50°C for 4 h and stored in a vacuum dryer [22].

The surface morphology and particle size of the PAL, nZVI and PAL-nZVI samples was observed with scanning electron microscopy (SEM, Zeiss Supra 55VP, Germany). The function groups on the surface of materials were obtained by fourier transform infrared spectroscopy (FT-IR, VERTEX 70, Germany) over $4000\text{--}400\text{ cm}^{-1}$, 32 scans were taken.

Remediation of Cd and Pb-Contaminated Soil

The remediation experiments were performed with 500 g contaminated soil in a plastic pot with a constant moisture content of 70% at room temperature ($25 \pm 2^\circ\text{C}$). In order to assess the dosage of PAL-nZVI on Cd and Pb stabilization efficiency, 5 g/kg, 20 g/kg, 40 g/kg, 60 g/kg and 80 g/kg of PAL-nZVI (0.5%, 2%, 4%, 6%, 8% w/w) were applied in the soil samples. The mixtures were incubated for 15, 30, 45 days to assess the reaction time on Cd and Pb stabilization efficiency. After 15, 30 and 45 days of incubation, 100 g subsamples from each pot were carried out to analyze the physicochemical

properties and bioinformatics of soil. Meanwhile, the contaminated soil sample without adding PAL-nZVI was used as the control (CK).

CaCl_2 Extraction

The effect of PAL-nZVI at different dosage and treatment time on the bioavailability of heavy metals was assessed by 0.01 M CaCl_2 . Briefly, 1 g dried soil samples were reacted with 25 mL of 0.01 M CaCl_2 solution at 25°C and shook for 120 min. After extraction, the samples were centrifuged at 3000 r/min for 2 min and the supernatant filtered the $0.45\text{ }\mu\text{m}$ membrane before to measure the Cd and Pb concentration by AAS.

Toxicity Characteristic Leaching Procedure (TCLP) Text

In order to evaluate the potential mobility and availability of Cd and Pb in the contaminated soil, the toxicity characteristics leaching procedure (TCLP), which was based on the US EPA Method 1311 (1992) was adopted. Briefly, measured accurately 5.7 mL glacial acetic acid in a 500 mL volumetric flask and added the deionized water to scale-line as a leaching solution and used 1 mol/L HNO_3 or 1 mol/L NaOH dropwise to regulate the pH as 2.88 ± 0.01 . Weighed 1.0 g dried soil sample and extracted using 20 mL leaching solution at 25°C in a 50 mL centrifuge tube and was shaken at 30 r/min for 18 h. Then centrifuged the slurry at 3000 r/min for 2 min after extraction and filtered the supernatant to the $0.45\text{ }\mu\text{m}$ membrane. Finally, using the AAS to determine the concentration of Cd and Pb in the supernatant.

BCR Sequential Extraction

The toxicity and bioavailability of heavy metals are closer dependent upon their speciation than their total

contents in soil. The European Community Bureau of Reference (BCR) four-step sequential extraction was adopted to determine the PAL-nZVI at different dosage and treatment time on the change of the Cd and Pb speciation in soil. It partitioned the heavy metals into four fractions: acid exchangeable fraction, reducible fraction, oxidizable fraction and residual fraction. The recovery rate of Cd and Pb in this test ranged from 94.95% to 110.35% and 92.55% to 101.74%, respectively. After each extraction, the acidified solutions were filtered through 0.45 μm membrane to measure the content of Cd and Pb in each fraction by AAS.

The potential ecological risk index (E_r) was used to evaluate the ecological risk from heavy metals in the soil and the index can be calculated as the following formulas according to [23]:

$$E_r^i = T_r^i \times \frac{C_d^i}{C_r^i} \quad (3)$$

...where T_r^i is the toxic-response factor for heavy metal and the Cd = 30, Pb = 5, C_d^i is the present concentration of heavy metals in soil, C_r^i is the national criteria (China) for heavy metal in soil and the Cd = 0.45 mg/kg, Pb = 250 mg/kg. The evaluation standard for E_r^i index is shown in Table 1.

Pot Experience

The PAL-nZVI was applied in the contaminated soil at the rate of 0, 0.5, 2, 4, 6, 8% (w/w). In each pot, 1.5 kg soil and PAL-nZVI mixture was added with three replications of each treatment. Incubated per treatment for 30 days with a constant moisture content of 70% at room temperature ($25 \pm 2^\circ\text{C}$), then planted 10 corn seeds in each pot. After 50 days, the shoot and root part of the corn was harvested using stainless steel scissors, respectively. Using the deionized water to rinse the corn's shoot and root part, measured their length by ruler and weighted the fresh weight of harvested plant material. The plant samples were put in the drying oven at 75°C for 48 h to let them dry thoroughly and obtained the dry-weight of samples. Then the dried plant samples were ground and sieved through a 0.149 mm mesh to measure the uptake of Cd and Pb in the corn's shoot and root part, respectively. Briefly, adding the 15 mL HNO_3 and 1 mL HClO_4 to 0.2000 g (dry weight) plant sample in the teflon crucible to make the wet digestion. Then filtered the digested solution to the 0.45 μm membrane and diluted to 50 mL ultrapure water in a volumetric flask and determine the concentration of total Cd and Pb by AAS.

In order to further explore the efficiency of PAL-nZVI remediation on the translocation capability of Cd and Pb for corn, the bioconcentration factors (BCF) and translocation factor (TF) were calculated as follows [35-36]:

$$\text{Bioconcentration factors (BCF)} = \frac{\text{heavy mealtals in the shoot or root parts of plants (mg/kg)}}{\text{heavy mealtals in the soil (mg/kg)}} \quad (4)$$

$$\text{Transfer factor (TF)} = \frac{\text{heavy mealtals in the aboveground parts of plants (mg/kg)}}{\text{heavy mealtals in the root of plants (mg/kg)}} \quad (5)$$

Statistical Analysis

The data set were subjected using the one-way ANOVA to analyze their variance, which processed using the SPSS 18.0 (IBM, USA) for Windows. Using the least significant difference (LSD) method to determine the significant difference among treatments and assign the same letter on the top of the histogram ($P < 0.05$).

The immobilization efficiency (IE) of Cd and Pb were calculated as follows [20]:

$$\text{IE}(\%) = (1 - C_{\text{PAL-nZVI}}/C_{\text{CK}}) \times 100\% \quad (6)$$

...where $C_{\text{PAL-nZVI}}$ is the Cd/Pb concentration under various extraction methods when the contaminated soil was amended by PAL-nZVI; C_{CK} is the Cd/Pb concentration under various extraction methods in control tests without any amendment.

Results and Discussion

Characterization of the Material

The SEM images of PAL, nZVI, PAL-nZVI were shown in Fig. 2(a-c) which could display the surface morphology of them. We could see clearly from Fig. 2b), because of the magnetism and Vander Waals forces, the bare nZVI had an apparent aggregation and presented the chain-like structures, which was consistent with Li et al. found [37]. After loading the nZVI with palygorskite, the surface of PAL turned rougher obviously shown from Fig. 2a) and c), which indicated that the metal(loid) ions had been captured on the surface or pores of palygorskite successfully. As shown in Fig. 2c), the spherical nanoparticles (approximately 40-60 nm) distributed homogeneously on the surface of multilayered palygorskite.

Table 1. Evaluation standard for E_r^i index [18].

<40	40-80	80-160	160-320	>320
Low risk	Moderate risk	Considerable risk	High risk	Very high risk

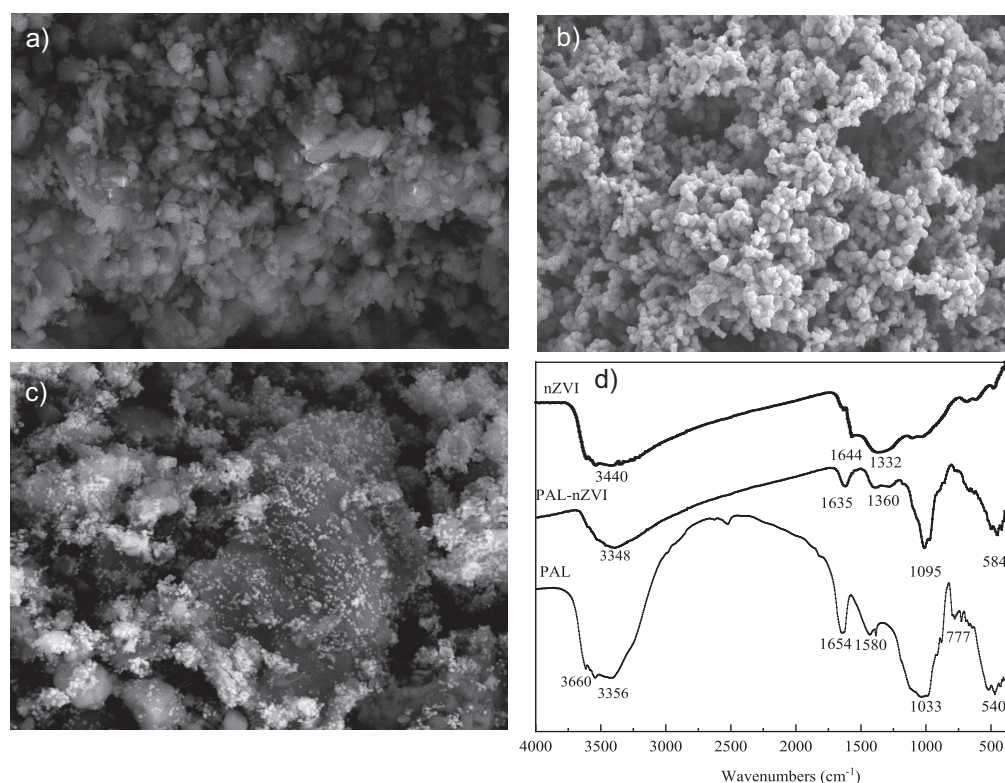


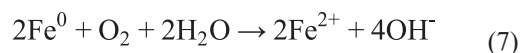
Fig. 2. SEM images of a) palygorskite, b) nZVI, c) PAL-nZVI, d) FTIR spectra of samples.

Fig. 2d) presents the FTIR spectra of PAL, nZVI, PAL-nZVI, which could reveal the functional groups of these materials. The O-H stretching vibration peak at 3348 cm^{-1} in PAL-nZVI was indicated that the hydrogen bonds had occurred between palygorskite and nZVI and the Si-OH and Fe-OH existed on the PAL-nZVI surface. Peaks at 3440 cm^{-1} of nZVI result from the formation of FeOOH on the surface of nZVI [35]. The peak at $1620\text{--}1644\text{ cm}^{-1}$ associated with the stretching vibration in the adsorbed water molecules. The iron oxyhydroxide had formed on the surface of nZVI and PAL-nZVI, the distinctive stretching vibration peak at $1360\text{--}1332\text{ cm}^{-1}$ could detect in nZVI and PAL-nZVI but were not detected in palygorskite [37]. The peak at 1033 , 777 and 540 cm^{-1} could result from stretching and transformation of Si-O-Si, Al-O-Si in palygorskite, which were changed for the weaker bonds of Si-O-Fe and FeOOH in PAL-nZVI. Consequently, plenty of functional groups, such as hydroxyl, Fe-O and FeOOH were generated on the surface of PAL-nZVI, which could supply a large number of active sites for the complexation of heavy metals.

Changes of Physicochemical Properties

pH played an essential role in controlling the transfer behavior of heavy metals in soil [38]. We could see from Fig. 3a) that when the dosage of PAL-nZVI was 0.5% and 2%, the change of soil pH was not obvious, while with the further increase of dosage, the pH of the soil increased significantly. When the dosage of PAL-nZVI

was 4%, 6% and 8%, the pH value of soil increased from 4.68 ± 0.02 to 5.24 ± 0.02 , 5.89 ± 0.02 and 6.05 ± 0.01 after 15 days, respectively. After 30 days, the soil pH increased to 6.02 ± 0.02 , 6.42 ± 0.02 , 6.49 ± 0.01 , which increased by 1.30, 1.70, 1.77 pH units than CK. After 45 days, the soil pH increased to 6.21 ± 0.01 , 6.47 ± 0.02 , 6.50 ± 0.02 , which increased by 1.54, 1.80, 1.83 pH units than CK. In previous research, when added the nZVI to co-contaminated soil, the soil pH increased by only 0.41 pH units after 42 days [23]. It could be seen that PAL-nZVI can improve the soil pH significantly and reach a stable station after 30 days. The reason why the pH value of soil increased was attributed to the corrosion of Fe^0 [35]. When the nZVI was oxidation as the following reaction, the OH^- ions were released, and the pH of system was increased significantly.



When the pH increased, the competition between H^+ and metal ions became weaker, which was easier for heavy metals to combined into the stable form (such as $\text{Cd}(\text{OH})^+$, $\text{Cd}_2(\text{OH})^{3+}$, $\text{Pb}(\text{OH})^+$, $\text{Pb}_2(\text{OH})^{3+}$) on the surface of iron (oxyhydro) oxide, therefore entrapment the Cd and Pb on the surface of PAL-nZVI particles.

As we all know, soil CEC was associated with the negative charge in soil. The more negatively charged sorption sites on the soil, the greater the soil CEC. At high soil pH, more negative charges had been carried on the surface of nZVI, and the CEC was increased with the application of PAL-nZVI. From Fig. 3b), after

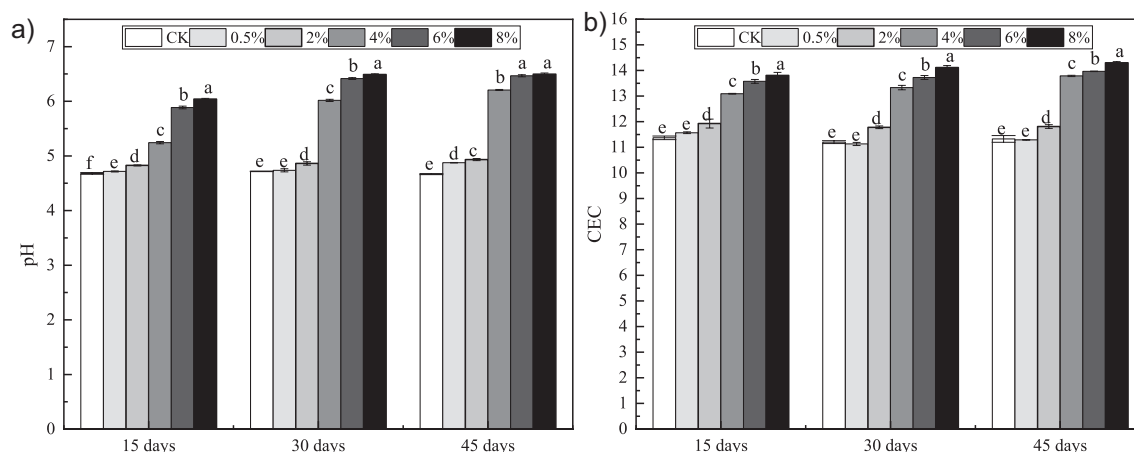


Fig. 3. The change of soil pH a) and CEC b) under different dosage of PAL-nZVI at various treatment time. Error bars indicate standard deviation (n = 3).

15 days, the CEC increased by 1.73%, 4.90%, 15.13%, 19.38%, 21.40% treated by PAL-nZVI (0.5%, 2%, 4%, 6%, 8%) than CK. After 45 days, the CEC increased by 0.35%, 4.24%, 21.68%, 23.24%, 26.21% treated by PAL-nZVI (0.5%, 2%, 4%, 6%, 8%) than CK. This finding was similar to that of Zhai [39], who reported that the soil CEC had a positive correlation between pH and could alleviate the toxic effect of heavy metals in soil.

CaCl₂-Extractable Cd and Pb

Fig. 4 showed the effect of remediation time and the amendment dosage on CaCl₂-extractable Cd and Pb. We found from Fig. 4a) and b), that when the soil samples were treated with PAL-nZVI for 30 days, the CaCl₂-extractable Cd decreased from its original 3.11±0.07 mg/kg to 2.80±0.00, 2.58±0.09, 1.32±0.15, 1.30±0.04, 1.29±0.03 mg/kg when the dosage was 0.5%, 2%, 4%, 6% and 8%, respectively. The immobilization efficiency increased to 57.66%, 58.20%, 58.63% when the dosage was 4%, 6% and 8%, respectively after 30 days of incubation. The immobilization efficiency was increased by 35.00%, 22.87%, 21.20% from 15th to 30th days, and increased by only 1.38%, 4.76%, 5.60% from 30th to 45th days when the dosage was 4%, 6% and 8%, respectively.

From Fig. 4c) and d), we could see that the concentration of CaCl₂-extractable Pb in the untreated soil was 4.72 mg/kg. With the increasing dosage of PAL-nZVI, the concentrations of CaCl₂-extractable Pb were reduced significantly. When 4%, 6% and 8% PAL-nZVI was applied into the soil, the CaCl₂-extractable Pb decreased from its original 59.43±4.88 mg/kg to 8.23±0.53, 6.73±0.31, 6.89±0.05 mg/kg, respectively, and the immobilization efficiency increased to 86.16%, 88.67%, 88.76% at 30 days. After 45 days, the immobilization efficiency had been nearly reached the equilibrium point and increased only 2.82%, 1.03%, 1.43% when the dosage was 4%, 6% and 8%, respectively.

With the increase of PAL-nZVI, the greater adsorption sites provided to heavy metals and tended to remain constant with that of the dosage of 8%. It was concluded that the 30 days was a critical period for Cd and Pb stabilization and the optimum dosage of PAL-nZVI was 6%. Li et al. used to apply the zeolite-supported nanoscale zero-valent iron to co-contaminated soil and the CaCl₂-extractable Cd and Pb reduced by 30% and 64%, respectively [10]. These results above suggested that the PAL-nZVI was an effective stabilizer for the bio-availability of Cd and Pb in multi-contaminated soil.

TCLP Method

The TCLP leachability concentration of Cd and Pb stipulated by the U.S. Environmental Protection Agency (USEPA1992) was 0.5 mg/L and 5 mg/L. As shown in Fig. 5, the TCLP leachability of Cd and Pb in the CK were detected to be 0.21 and 2.25 mg/L, which were both below the limits value of standard but still had the potential toxicity for the human beings. The TCLP leachability concentration of Cd and Pb were decreased significantly in the soil remediated with PAL-nZVI. The addition of PAL-nZVI at the dosage of 6%, markedly decreased the TCLP leachability concentration of Cd from 0.22±0.00 mg/L to 0.07±0.01 mg/L and Pb from 2.53±0.04 mg/L to 1.12±0.05 mg/L at 30 days. The immobilization efficiency increased by 66.98% and 48.39% than CK. This result could be explained by excess negative charges, small particle size, high mineral ash contents and a large number of functional groups on the surface of PAL-nZVI. Furthermore, the application of PAL-nZVI was proved more effective in reducing of TCLP Cd than Pb. It may because the Cd(OH)₂ had the low solubility relatively and high hydrolysis content of Cd²⁺ was exist in the soil, which could inhibit the Pb adsorption at the same active sites. It was in accordance with Fajardo, who reported that the nZVI could decrease the TCLP Cd and Pb in soil, particularly for Cd.

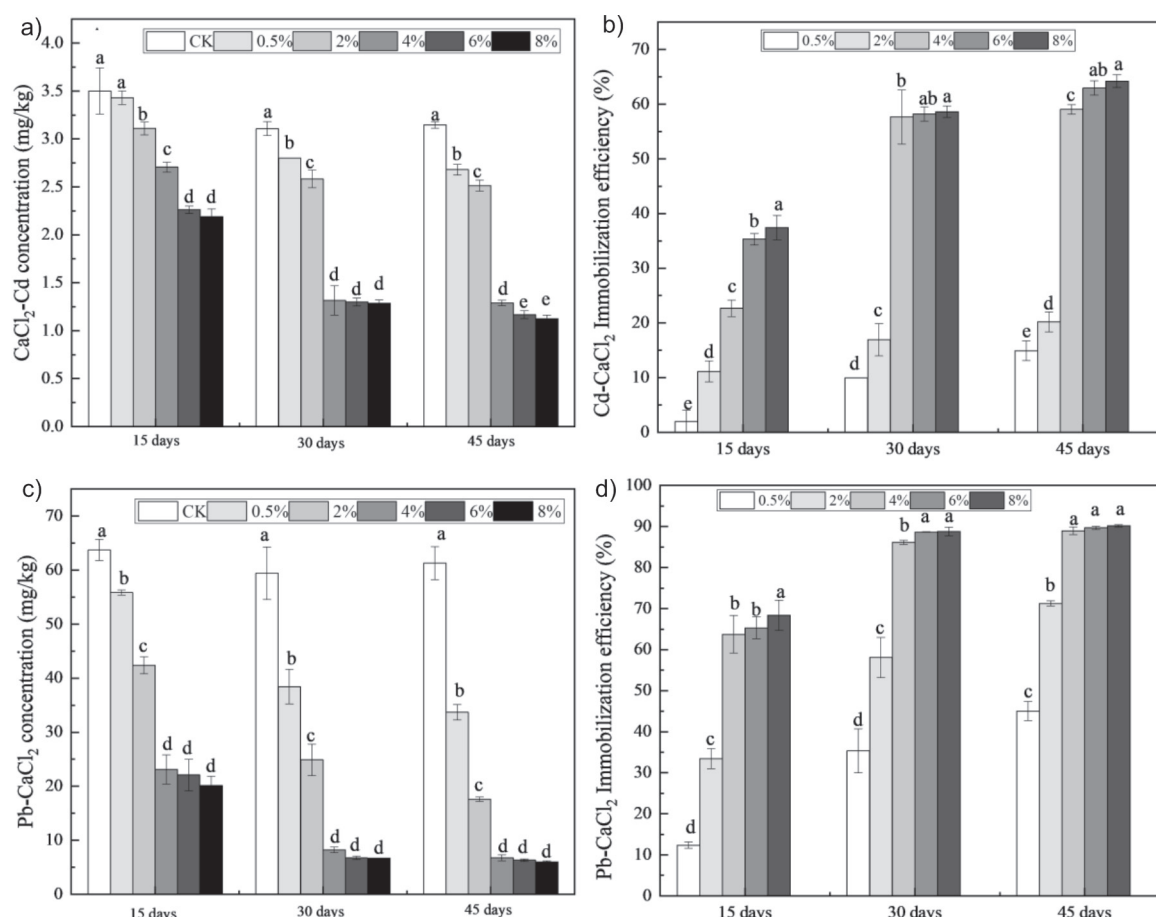


Fig. 4. The CaCl_2 -extractable Cd/Pb and immobilization efficiency at various dosage of PAL-nZVI when the soil samples were treated by the different treatment time. Error bars indicate the standard deviation ($n = 3$) and the same letters indicated the significant differences between treatments at $p < 0.05$.

Besides, the treatment time would also be an essential factor in increasing the immobilization efficiency of Cd and Pb. After PAL-nZVI was added into the soil, it needed some time to disperse in the soil and come into full contact with the heavy metals. Hence, it was necessary to prolong the stabilization time to enhance the immobilization efficiency of Cd and Pb [40]. When the dosage of PAL-nZVI increased to 8%, the TCLP leachability concentration of Cd and Pb tended to remain unchanged and the equilibrium was almost at 30 days. Obvious, the immobilization of Cd and Pb in contaminated soil was slow and complicated, which was unlike the fast adsorption process of heavy metals by nZVI or modified nZVI in aqueous solutions [5].

Speciation of Cd and Pb in Soil

We could see evidence that the speciation of Cd and Pb in soil changed greatly after stabilization (Fig. 6). From Fig. 6a), we found that the acid soluble fraction was the dominant speciation of Cd in the contaminated soil. It accounted for 64.28% of the total concentration in original soil. After 15 days of stabilization, the acid soluble fraction decreased by 2.35%, 7.40%, 11.55%,

18.21%, 21.76% than CK, decreased by 4.74%, 12.00%, 22.01%, 29.50%, 29.92% than CK after 30 days, and decreased by 4.26%, 14.67%, 25.12%, 29.93%, 32.26% than CK after 45 days when the dosage of PAL-nZVI was 0.5%, 2%, 4%, 6%, 8%, respectively. We could see that after 30 days, the change of Cd speciation tended to stabilize. Compared to CK, the percentage of residual fraction increased from 13.50% to 15.45%, 24.51%, 34.23%, 40.74%, 40.84% with the treatment of PAL-nZVI (0.5%, 2%, 4%, 6%, 8%) after 30 days of stabilization, respectively, which indicated that the toxicity of the Cd alleviated directly [23]. For Pb, as shown in Fig. 6b), it was mainly distributed in the reducible fraction in the contaminated soil, which took up to 74.07% in CK. The residual fraction of Pb increased by 4.93%, 17.78%, 28.93%, 33.75%, 36.57% than CK after 30 days of stabilization. Additionally, when the dosage of PAL-nZVI was 6% and 8%, the residual fraction of Cd and Pb both increased significantly and simultaneously. Consider the cost of remediation and the introduction of exotic Fe into the soil, so we choose the 6% of PAL-nZVI as the optimum dosing. We could demonstrate clearly that the Cd and Pb both converted from the active speciation to stable

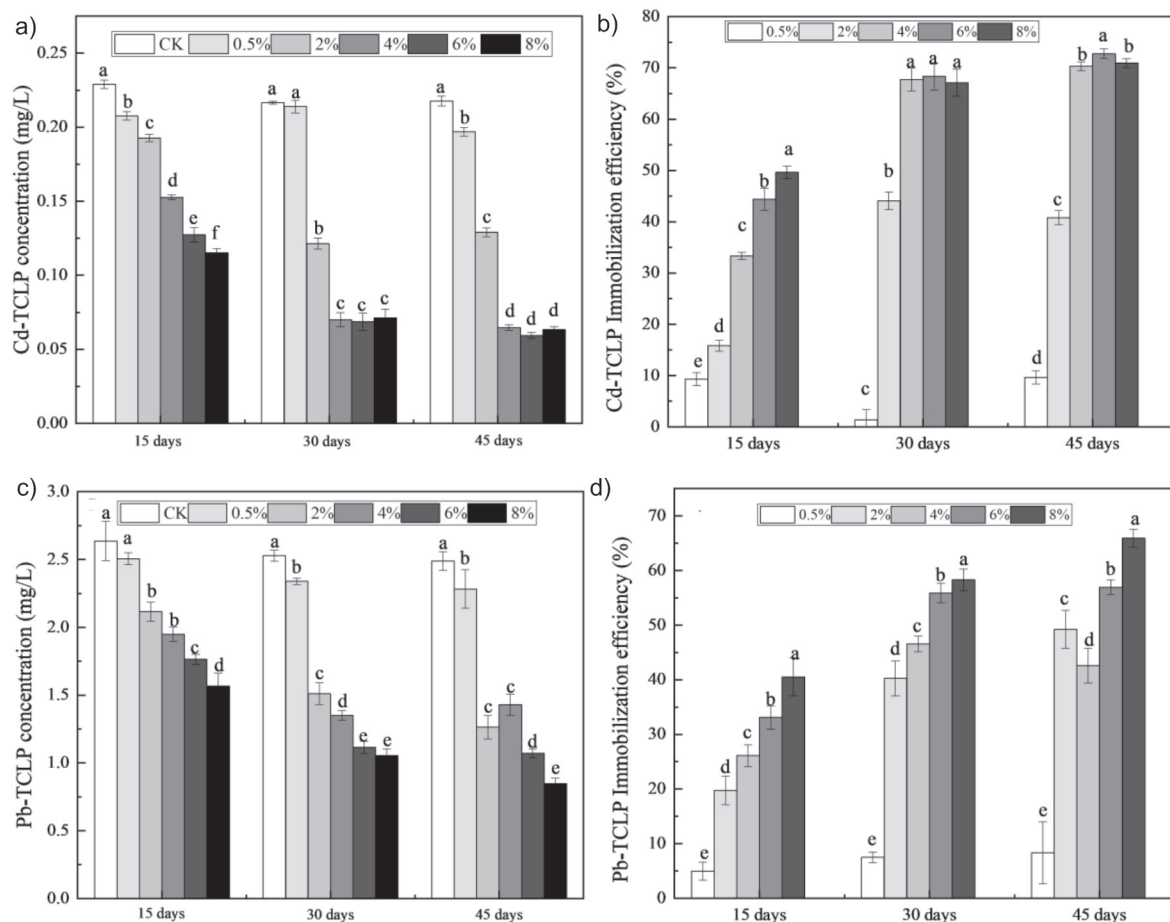
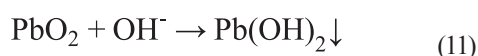
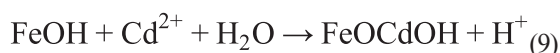


Fig. 5. The TCLP leachability concentration of Cd/Pb and immobilization efficiency at various dosage of PAL-nZVI when the soil samples were treated by the different treatment time. Error bars indicate the standard deviation ($n = 3$) and the same letters indicated the significant differences between treatments at $p < 0.05$.

speciation after adding the PAL-nZVI in the soil. This phenomenon could explain as follow: after applying the PAL-nZVI, a layer of the iron oxyhydroxide (FeOOH) could be formed on the surface of PAL-nZVI due to the oxidation of iron, which provided the active sites to complexing with Cd in the soil. Meanwhile, Pb (II) should be precipitated to form the Pb (OH)₂ after be oxidized. The reaction could occur via the following equations [23]:



The formed FeOCd⁺, FeOCdOH, Pb (OH)₂ were relatively stable and could be regarded as the residual fraction, which would be adsorbed and utilized difficultly by biology [20]. What's more, H⁺ appeared during the reaction between FeOOH and Cd (Eq. (9)),

the surface -OH might be protonated to form OH₂⁺, which could provide more and stronger active sites to adsorb the heavy metals. On the other hand, due to the difference in the standard potential between lead (-0.13 V) and zero-valent iron (-0.41 V), the mechanism of Pb (II) stabilized by nZVI included the direct reduction in addition to adsorption and the formation of complexes. The standard potential of cadmium is -0.40 V, which is very close to that of Fe⁰. Therefore, the mechanism of Cd (II) stabilized by nZVI consist of the sorption and complex formation [17]. Furthermore, a large number of functional groups (such as Si-O, C=O) also composed the structure of palygoskite, which could form the ligand with Cd and Pb in the contaminated soil [26].

The potential risk of heavy metals was also influenced by the distribution of their speciation in the soil. When the metal turned from an active fraction to a stable fraction, the risk that the metal released to the environment weakened considerably, and the metals would hardly remove from soils. According to the potential ecological risk index shown in Table 2, we could see that in this co-contaminated soil, Cd posed a very high risk to the environment. After 15 days, E_r^{Cd}

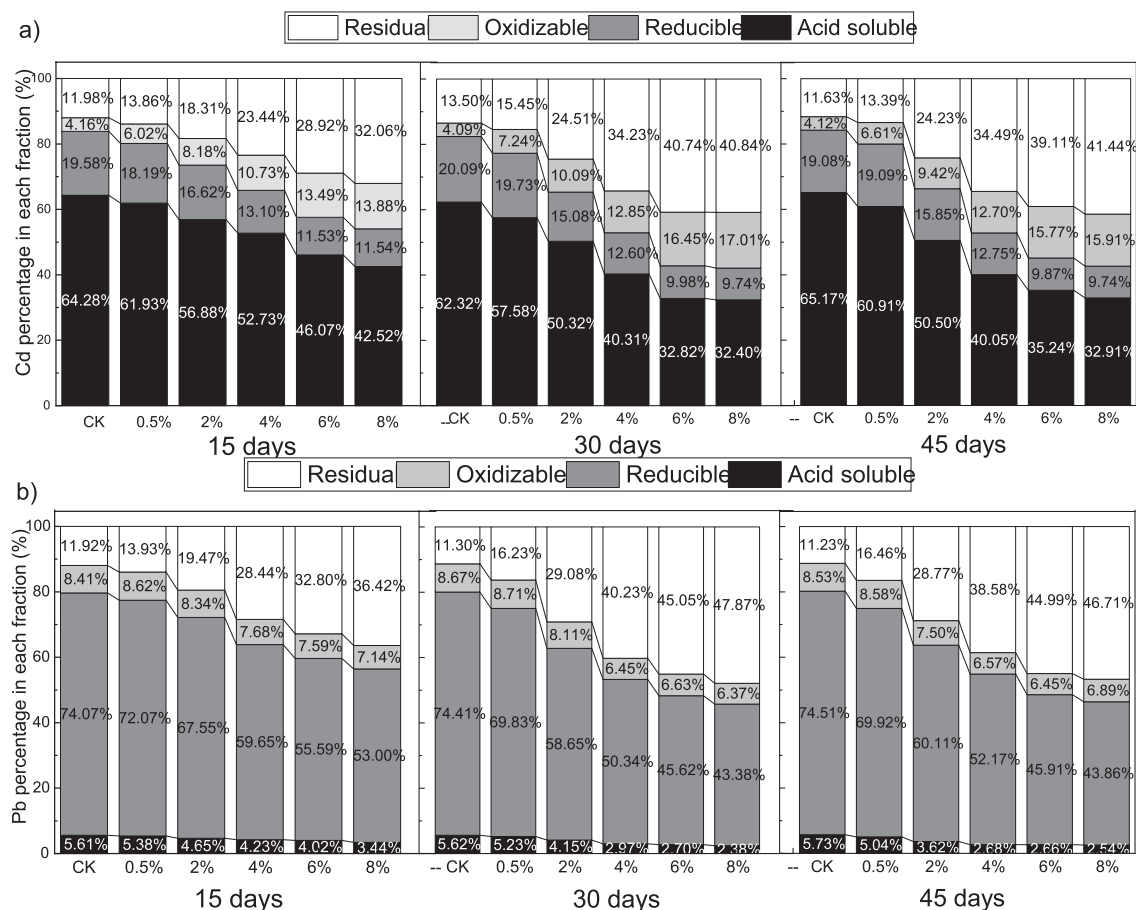


Fig. 6. The speciation of Cd and Pb in soil at various dosage of PAL-nZVI when the soil samples were treated by the different treatment time. a) Speciation of Cd; b) Speciation of Pb.

index reduced by 28.79% and 30.40% when the dosage of PAL-nZVI increased to 6% and 8%, respectively. After 30 days, E_r^{Cd} index was reduced by 35.13% and 33.77% when the dosage of PAL-nZVI increased to 6% and 8%, respectively. After 45 days, E_r^{Cd} index was reduced by 37.15% and 39.34% when the dosage of PAL-nZVI increased to 6% and 8%, respectively. In addition, Pb had a low potential ecological risk to

the environment. In the original soil, E_r^{Pb} index was around 5.2, while it could drop below 3.0 after adding the PAL-nZVI. The same results were reported by Cao [18]. Although the total Cd and Pb concentration in soil did not change, while after adding the PAL-nZVI, the residual fraction increased and the sum of acid soluble, reducible and oxidation fraction of Cd and Pb decreased. The release risk of Cd and Pb to the

Table 2. Potential risk (E_r) of Cd and Pb at various dosage of PAL-nZVI when the soil samples were treated by the different treatment time.

Days	E_r	PAL-nZVI dose in %					
		0	0.5	2	4	6	8
15	Cd	1203.11±16.20 a	1199.96±6.97 a	1099.62±11.21 b	994.84±14.17 c	856.69±9.82 d	837.38±21.70 d
	Pb	5.25±0.03 a	4.95±0.10 b	4.71±0.03 c	4.29±0.08 d	3.92±0.05 e	3.67±0.00 f
30	Cd	1183.24±30.05 a	1110.56±13.60 b	1051.73±3.59 c	906.73±27.78 d	767.51±27.11 e	783.67±11.48 e
	Pb	5.22±0.03 a	4.83±0.12 b	4.00±0.02 c	3.56±0.07 d	3.15±0.07 e	2.91±0.08 f
45	Cd	1237.58±17.43 a	1176.38±2.89 b	985.02±22.25 c	842.49±6.48 d	777.80±26.17 e	750.71±16.45 e
	Pb	5.28±0.02 a	4.71±0.08 b	4.22±0.08 c	3.61±0.07 d	3.11±0.06 e	2.90±0.06 f

E_r^{Cd} and E_r^{Pb} were calculated by the Eq. 3. The non-residual metal fraction content (sum of acid soluble, reducible and oxidation fraction of the Cd and Pb) in soils were chosen as the C_r^{Cd} and C_r^{Pb} .

environment was weakened dramatically by reducing the concentration of labile metal speciation and increasing the strongly bound speciation.

Plant Growth and Metal Bioavailability

Effects on Plant Growth

The effect of PAL-nZVI on the growth situation of corn was shown in Fig. 7. PAL-nZVI could significantly improve the growth of the plant. The length, fresh weight and dry weight of corn all increased simultaneously, and the more PAL-nZVI that was applied, the better the corn grew. Compared with the CK, the corn's length of root increased by 228.48%, 222.76%, 234.19%, and the corn's length of shoot increased by 118.78%, 107.84%, 120.35% when the dosage of PAL-nZVI was 4%, 6%, 8%, respectively. The fresh weight of corn increased by 33.87%, 65.58%, 169.12%, 189.99% and 185.68% with

the addition of 0.5, 2, 4, 6, 8% PAL-nZVI, respectively. The dry weight of corn had the same increase trend, and the growth rate up to 169.2% compared with CK when the dosage of PAL-nZVI was 6%. It could be seen that when the addition of PAL-nZVI was great than 4% in contaminated soil, it would have an obvious promoting effect on the growth of plants. However, when the addition of PAL-nZVI increased from 6% to 8%, the biomass of corn increased not significant. The plant's resistance to the Cd and Pb stress in soil was enhanced when applied the PAL-nZVI in contaminated soil, and the stress generated by the toxicity of heavy metals was decreased for the plant.

Accumulation and Translocation of Cd and Pb by Plant

The remediation effect of the amendment for Cd and Pb-contaminated soil was aimed at decreasing

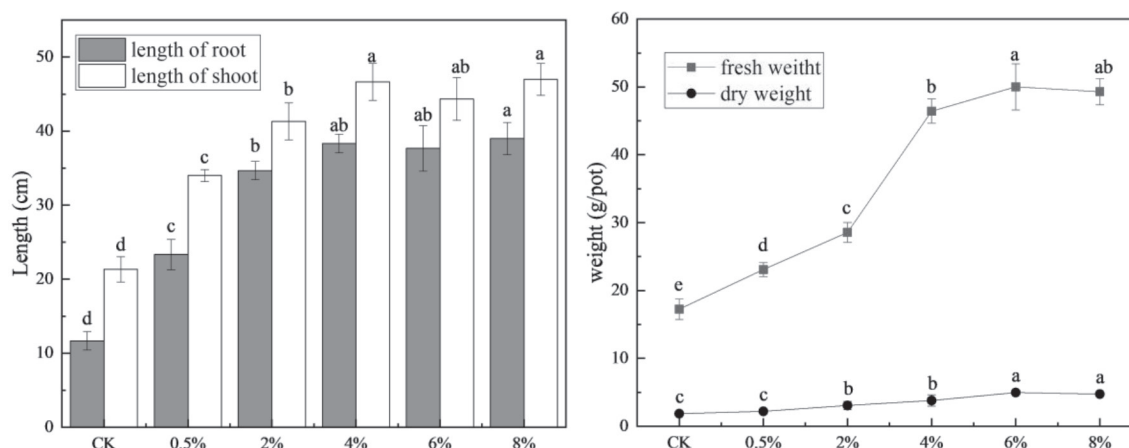


Fig. 7. The length and biomass of shoot and root part of corn grown in the soil amended with the various dosage of PAL-nZVI. The different letter (a, b, c) within the same column indicate significant differences at $P < 0.05$.

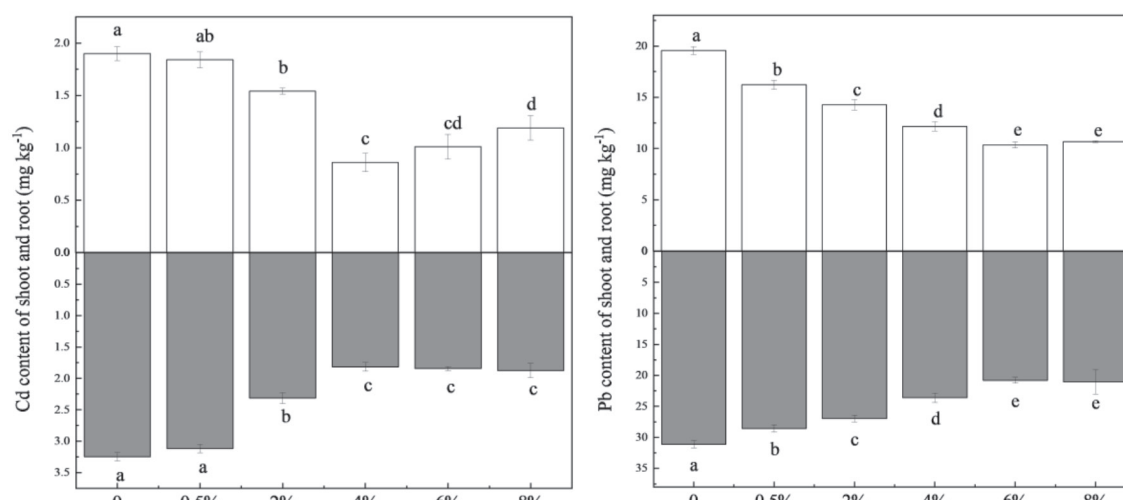


Fig. 8. Cd and Pb contents of roots (lower part) and shoots (upper part) at various dosage of PAL-nZVI. Data are means \pm SD ($n = 3$). Bars with different letter (a, b, c) within the same column indicate significant differences at $P < 0.05$.

Table 3. BCF and TF of Cd and Pb in plants at various dosage of PAL-nZVI.

PAL-nZVI dose in %	Cd			Pb		
	BCF _{shoot}	BCF _{root}	TF	BCF _{shoot}	BCF _{root}	TF
0	0.099±0.003 a	0.171±0.004 a	0.586±0.011 ab	0.208±0.004 a	0.330±0.006 a	0.629±0.002 a
0.5	0.097±0.004 a	0.164±0.004 a	0.590±0.013 ab	0.172±0.005 b	0.303±0.006 b	0.568±0.008 b
2	0.081±0.001b	0.121±0.005 b	0.666±0.014 ab	0.151±0.005 c	0.286±0.006 b	0.529±0.029 bc
4	0.045±0.005 d	0.095±0.004 c	0.472±0.042b	0.129±0.005 d	0.251±0.008 c	0.515±0.034 bc
6	0.053±0.006 cd	0.097±0.002 c	0.548±0.055 ab	0.110±0.003 e	0.221±0.005 d	0.498±0.005 c
8	0.063±0.006 c	0.099±0.006 c	0.634±0.101 a	0.113±0.001 e	0.224±0.021 d	0.506±0.049 bc

the accumulation and migration of Cd and Pb by the plant. To further estimate the stabilization efficiency of the PAL-nZVI on Cd and Pb in soil, the uptake and translocation of Cd and Pb in the corn grown in contaminated soil were measured. As shown in Fig. 8, the accumulation of Cd and Pb by corn reduced significantly compared to the CK when applied the PAL-nZVI in soil. The concentration of Cd in the shoot part of corn decreased by 3.16%, 18.95%, 54.91%, 46.84%, and 37.37%, respectively, and the root part of corn decreased by 4.1%, 28.82%, 44.10%, 43.28%, and 42.26%, respectively, compared to CK when the dosage of PAL-nZVI was 0.5%, 2%, 4%, 6%, 8%. Simultaneously, the content of Pb decreased by 47.11% in the shoot part and 33.12% in the root part of corn with the 6% addition of PAL-nZVI. Nevertheless, adding more than 6% PAL-nZVI could not further decrease the Cd and Pb accumulation in the plant.

Table 3 showed the BCF of the shoot and root part and the TF calculated by Eq. 4 and 5. The BCF_{shoot} and BCF_{root} of Cd declined by 47.0% and 43.3%, Pb declined by 47.1% and 33.0%, respectively when the dosage of PAL-nZVI was 6%. However, both the TF of Cd and Pb had only reduced slightly. These findings suggested that the PAL-nZVI remediation was efficient from the detoxification opinion [35].

Conclusions

In this study, palygorskite was used as a carrier for nZVI to synthesize a new type of stabilizer for Cd and Pb stabilization from co-contaminated soil. The nZVI could effectively reduce from being aggregated and oxidized after loaded with palygorskite. It distributed on the surface of palygorskite keeps a spherical nanoparticle with the diameter of 40-60 nm. The bioavailability and TCLP leachability of Cd and Pb in soil could be decreased significantly ($P < 0.05$) after it was stabilized by PAL-nZVI with the dosage of 6% after 30 days. Meanwhile, the speciation of Cd and Pb has partially transformed from an unstable fraction into a stable fraction, and the potential risk of Pb and Cd decreased significantly in

the soil after amended by PAL-nZVI. Adsorption, cation exchange and electrostatic action were the most likely mechanism of Cd immobilization and the reduction, adsorption, oxidation and precipitation were the most likely mechanism of Pb immobilization by PAL-nZVI in the soil. Additionally, adding the PAL-nZVI to the soil could promote the growth of corn and reduce its accumulation of Cd and Pb. Overall, the PAL-nZVI is a low-cost, eco-friendly and easy-available material that can be a feasible and potential amendment to remediate the Cd and Pb contaminated soil effectively.

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Conflict of Interest

The authors declare no conflict of interest.

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