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Immobilization of Pb and Cd in contaminated soil using nano-crystallite hydroxyapatite

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Abstract

The efficiency and mechanism of nano-hydroxyapatite (nHA) in immobilizing Pb and Cd in contaminated soil were assessed in this work. The X-ray diffraction analysis indicated that the key mechanisms for Pb/Cd immobilization in the soil involve both surface complexation on the surface of nHA and dissolution of the nHA amendments and precipitation of Pb/Cd-containing phosphates. We tested the effects of different nHA dose (the molar ratio of P/(Pb+Cd)) on the water extraction of heavy metals, and phytoavailability of soil metals. The nHA application could significantly reduce water soluble Pb with 72% and Cd with 90%, bioaccessible Pb with a reduction of 12.5-27.5% and Cd with a reduction of 17.66-34.64%, respectively. The nHA reduced phytoavailability of Pb and Cd with 65.3% and 64.6% in contaminated soil, respectively. In addition, the treatment efficiency increased along with the nHA dose (based on the molar ratio of P/(Pb+Cd)) increased, and most effective ratio was molar ratio of P/(Pb+Cd) at 5:1. The results in this study showed that nHA can immobilize Pb and Cd in contaminated soil effectively.

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1. Introduction

Soil contamination with heavy metals is a worldwide problem. Accumulation of heavy metals in soils can adversely affect soil ecology, agricultural productivity, quality of agricultural products and water resources, human and animal serious health problem [1]. Lead (Pb) and cadmium (Cd) are ubiquitous heavy metal pollutants in soils due to their use widely. The primary sources of Pb contamination include industrial activities such as mining, smelting of metals, and the use of Pb-containing products such as paints, lead-acid batteries, bullets, gasoline and pesticides [2, 3]. It can damage human nervous (especially children) and reproductive systems (agency for toxic substances and disease registry) [4].

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Cadmium has been widely used in batteries, metal plating, plastic, nonferrous mining and the cause of Itai-Itai disease [3]. The high concentration of Pb and Cd in the soil poses risks to human and animal health by the leaching of metals from the soil into water and the consumption of edible plants grown in the contaminated soil. Therefore, proper remediation is necessary to reduce metal availability in soil for protecting human health.

Among available remediation technologies, in situ immobilization of heavy metals using a chemical amendment can be a cost-effective and environmentally sustainable remediation approach for the immobilization of heavy metals by reducing the mobility and availability. This immobilization technique may provide a long-term remediation solution if low solubility minerals and/or stable precipitates are produced in situ [5]. Therefore, the choice of the soil amendments need that the amendments must reduce heavy metals transfers from contaminated soils to the surface water or groundwater and uptake by plants and organisms. Up to now, soil amendments, for example lime [6, 7], organic matter [8], calcium carbonate [9, 10], red-mud [11], bone meal [10, 12], fly ash [10] and so on, added to the contaminant soil may decrease leachable concentrations of contaminants and thus reduce the detrimental effects of heavy metals on environmental receptors, such as microorganisms, plants, animals, water bodies and humans [13].

Amounts of works have been made to evaluate the effectiveness of phosphates on in situ immobilization of contaminated water and soils [2, 3, 14-19]. The mechanisms include precipitation, surface complexation, adsorption, dissolution of apatite followed by coprecipitation, or an ion exchange process at the surface of phosphate minerals. Owing to nano-material with higher reactivity and sorption ability than the same material of normal size, some nano-material had been used for soil amendment with high heavy metal concentration. Nano-hydroxyapatite (nHA) is a less soluble phosphate, and would be an ideal material for the immobilization of heavy metals because of its high sorption capacity for heavy metal, low water solubility, high stability under reducing and oxidizing conditions, availability and cost-effect. However, there is limited information about heavy metal immobilization by nano-hydroxyapatite [3, 14], especially in soil. The purpose of this study was to evaluate the effectiveness of nano-hydroxyapatite in immobilizing Pb and Cd in contaminated soil. The specific objectives were to: (1) determine nHA-induced metal transformation in soil using X-ray diffraction, scanning electron microscopy and sequential extraction method; (2) estimate the availability of Pb and Cd after nHA-induced using water extraction, plant metals uptake, and a simple Physiologically Based Extraction Test (PBET) [11, 20].

2. Materials and methods

2.1. Experimental materials

Soil was collected from the surface layer (0-20cm) of agricultural land in Duling, south Xi'an, China. After air-dried, the soil sample was passed through a 2mm sieve. The physico-chemical properties of the soil were given in Table 1.

Table 1. Selected physicochemical properties of the soil.

	pH	CEC	OM	Sand (%)	Silt (%)	Clay (%)	Pb (mg kg ⁻¹)	Cd (mg kg ⁻¹)
soil	6.0	12.6	1.58	52.3	25.4	31.7	20.6	0.097

The soils contaminated by Pb and Cd were prepared by artificially mixed in this research. According to the polluted condition in soils near smelter plant, the Pb and Cd concentration in experimental soil were determined as 800mg Pb and 40mg Cd per kilogram air-dried soil, respectively. The dosages of Pb

and Cd were calculated by lead nitrate and cadmium nitrate. The homogenous mixed lead nitrate and cadmium nitrate amount with 1.3 kg soils was loaded in plastic pot (15cm diameter,18cm height), and was then equilibrated for two month with frequent thorough mixing at room temperature.

Nano-hydroxyapatite (nHA) used in this study (purity 99.9%) was purchased from DK nano-material company (Beijing, China).The chemical composition and the specific surface area of the nHA were in Table. 2. The crystalline structure and particle size of nHA were determined with X-ray Diffraction (Rigaku, Ultima IV, Japan) and Scan Electron Microscopy (SEM, JSM-6510LV), respectively.The average particle size of nHA was 20 nm. TEM results illustrated that the nHA material was nano-rod structure, with dimensions of 40 nm (wide)×200 nm (length). The Ca/P molar ratio of nHA was 1.67 using XRF methods. The specific surface area of nHA was calculated as 50 m² g⁻¹ by the BET method.

Table 2. Chemical composition and specific surface area of HA.

	Sulfate (W %)	Chloride (W %)	Heavy metals	Mg (W %)	Na (W %)	Fe (W %)	Al (W %)	Ca/P	specific surface area (m ² g ⁻¹)
HA	0.035	0.02	6.5ppm	0.4	0.1	0.07	0.03	1.67	50

2.2. Pb/Cd immobilization experiment

NHA was applied to soils at molar ratios of P/(Pb+Cd) such as 2:1, 3:1, 4:1 and 5:1. Non-nHA soil was used as the control. The nHA was thoroughly mixed with the soil to obtain homogeneity, and was then equilibrated for 60 d, during which soil moisture was maintained with 16-18% (mass percent), and the soils were thoroughly mixed at weekly intervals. Digestion of soil sample was performed using HCl, HNO₃, HF and HClO₄ hot block digestion procedure [21]. Concentrations of Pb and Cd in the digested solution were measured using ICP-MS (PE, ELAN DRC-e).

2.3. Mineralogical characterization

To identify the mineral phases in soils, the clay fractions of selected soil samples were sieved, where the contaminant metals are more likely concentrated [22]. This metal-rich fraction was then characterized using X-ray diffraction (XRD, Ultima IV, Rigaku) with Cu K α radiation (40kv, 40mA) and PDXL2 software with ICDD (2012). Samples were scanned at room temperature by using start angle 10°, end angle 45°, step size 0.02° with a scanning speed 2° m⁻¹. Selected soil clay was also observed using a scanning electron microscopy (SEM, JSM-6510LV).

2.4. Water extraction Pb/Cd content and bioavailability test

The effect of nHA on contaminant extractability was evaluated using de-ionized water (DW) 2.5 g soil were added to 25mL DW in 50mL polypropylene centrifuge tubes, which were then shaken for 1h on a wrist-action shaker, centrifuged for 20 min at 1,1000×g, and then filtered through a 0.22- μ m pore-size Millipore filter.

The bioaccessibility to the human gastrointestinal (GI) system of Pb and Cd in ingested soil was measured using the modified physiologically based extraction test (PBST) procedure [24]. 0.5g soil was shaken (30rpm) with syntentic gastric solution (50ml 0.4 mol/L glycine, pH 2±0.5) for 1h at 37°C. When extraction was complete, the solution was immediately passed through at 0.22- μ m pore-site Millipore filter and measured using ICP-MS. Pb and Cd bioaccessibility were expressed as percentage of metal mass extracted by PBET with respect to the metal mass in soil.

2.5. Pb/Cd forms by sequential extraction

Sequential extraction method of Tessier was used in this study [23]. The extraction was carried out in 50 ml polypropylene centrifuge tubes with an initial mass of 0.5 g samples. The five heavy metal forms were divided in the following fractions: exchangeable form (EX), carbonate-bound form (CB), Fe/Mn oxide-bound form (OX), organic matter/sulfide-bound form (OM), residual form (RS). Sequential extraction scheme for Pb and Cd in contaminated soils as described in Table 3. After each successive extraction, separation was accomplished by centrifugation at 8000 r/min for 10 min.

Table 3. Sequential extraction scheme for Pb and Cd in contaminated-soils.

Step	Fraction	Extraction procedure
1	Exchangeable form	10ml 1M MgCl ₂ PH 7 (1h, 250rpm, room temperature)
2	Carbonate-bound form	15ml 1M CH ₃ COONa (PH 5with CH ₃ COOH 6h, 250rpm, room temperature)
3	Fe/Mn oxide-bound form	15ml 0.04M NH ₂ OH·HCl (PH 2 with CH ₃ COOH, 5h, 250rpm, 96 °C)
4	Organic matter/sulfide- bound form	5ml 0.02M HNO ₃ , 5ml 30%H ₂ O ₂ (2h, 85 °C) Then 5ml 30%H ₂ O ₂ (2h, 85 °C), Finally, 5ml 3.2M CH ₃ COONH ₄ in HNO ₃ (20%, V/V) for 0.5h at room temperature (250rpm)
5	Residual form	HCl, HNO ₃ , HF and HClO ₄ digestion

2.6. Pb/Cd Phytoavailability test

In order to examine the effect of the addition of nHA on the phytoavailability of heavy metals, corn was selected as experimental plant, prepared as described following: Corn seeds soaked in the water for 15 minutes initially, then tiled them into a plate and placed in the incubator (30 °C) for germination. After germination, the seedlings were transplanted into wet sawdust with daily watering. After 3 d, the corns grew to 5cm high approximately, then transplanted them into contaminated soils (2 plants/pot, 3 pots per treatment). The trials were conducted under controlled greenhouse conditions (temperature 25–35 °C, relative humidity 60–70%) with daily watering. After 40 d, the corns were harvested, rinsed with de-ionized water, and dried at 80 °C for a constant weight. Corn shoot samples were digested with HCl, HNO₃, HF and HClO₄, and the resultant solutions were filtered through a 0.22- μ m membrane filter, and analyzed for the heavy metals using ICP-MS.

2.7. Statistical analyses

Experimental data, including the Pb and Cd concentrations in soils and in corn shoots and plant biomass, were expressed as mean of three replicates. Statistical analyses were conducted using SPSS 11.5. Treatment effects were determined by analysis of variance of SPSS. Significance was tested at the 0.05 probability levels.

3. Results and discussion

3.1. Soil characterization after application nHA

The XRD analysis showed that no significant difference exist between nHA-treated soil and the control soil. However, two additional peaks at 2.89 Å ($2\theta=30.90^\circ$) and 3.19 Å ($2\theta=27.89^\circ$) were identified, which

may represent calcium lead phosphate hydroxide ($\text{Pb}_x\text{Ca}_{10-x}(\text{PO}_4)_6(\text{OH})_2$) by ICDD (2012) (Fig.1). It was consistent with the study of Laperche *et al.* that they may represent mixed Ca-Pb phosphates [24]. Furthermore, the intensity of peaks at 2.52\AA ($2\theta=35.53^\circ$), 2.49\AA ($2\theta=36.01^\circ$) and 3.77\AA ($2\theta=23.50^\circ$) increased in nHA-treated soil. They were lead dihydrogen phosphate, Pattersonite and lead phosphate, respectively. This showed that one of the mechanism of metals immobilization by nHA was the dissolution of HA and the precipitation of new metal phosphate minerals.

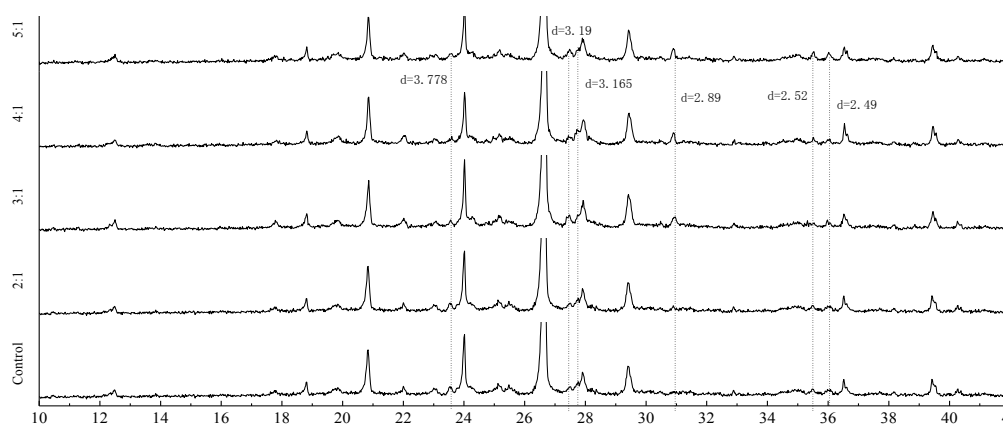
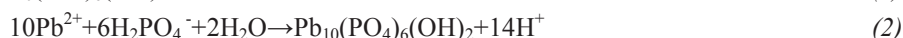
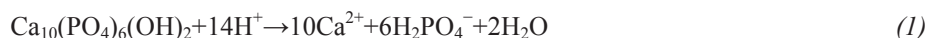


Fig. 1. X-ray diffraction patterns of nHA-treated soil and control soil at the end of experiment.

As was shown by Ma *et al.* [25, 26], the mechanism of Pb immobilization in the contaminated soils by HA could be dissolution of HA (eq.1), and the precipitation of hydroxypyromorphite (eq.2). However, Shaskova posed another reaction mechanism [27]. Pb may be adsorbed first on the surface of the HA and then cation exchange would prompt Pb^{2+} ions substituting Ca^{2+} ions in soil solution, following reaction (eq.3) [28]. Furthermore, as indicated by this equation, the mechanism is controlled by the amount of exchangeable cation.



For Cd, isomorphic substitution (the last mechanism) appears more significant than precipitation substitution [29]. Cd^{2+} was first adsorbed onto the nHA surface and then substitute the Ca^{2+} described by the following eq.4 [34].



This might be due to the fact that the ionic radius of Cd^{2+} (0.097 nm) is very close to that of Ca^{2+} (0.094 nm) [29]. But, no Cd minerals was detected by XRD (Fig.1), except peak at $2\theta=27.94^\circ$ (Cd, Pb hydroxylapatite) in the present study. This could be due to its relatively low concentration and the limited dissolution of the Cd-bearing minerals, which would reduce the formation of new phosphate phases [24, 31], or form some poorly crystalline mixed metal phosphates [32]. Besides, the precipitation of a new solid phase would need a long interaction time as reported by Cao who observed chloropyromorphite after 330 d of phosphate application [15]. SEM analysis showed that the soil particles after the nHA was added for 60 d present a smoother morphology than the un-treated soil (Fig. 2), which was consistent with Mignardi [19].

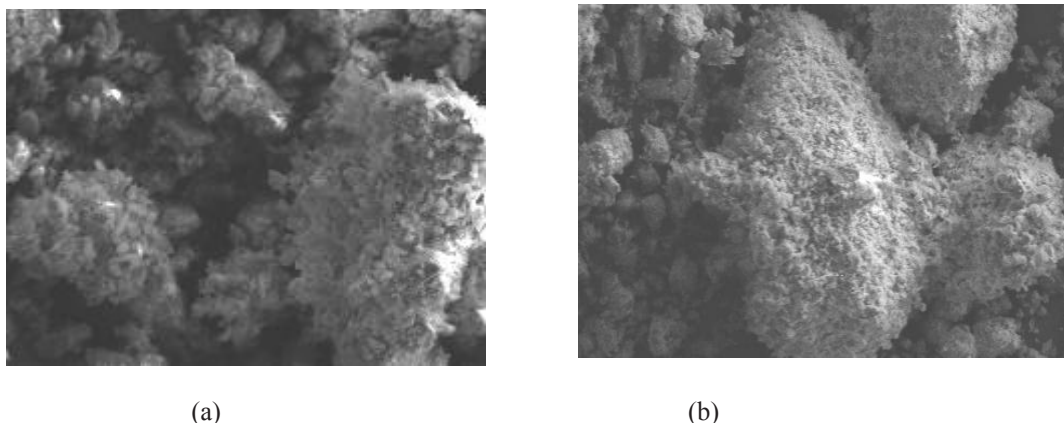


Fig. 2. Scanning electron microscopy images of control soil (a) and the nHA-treated soil(b) .

3.2. HA-induced Pb/Cd immobilization

Tessier sequential extraction has been extensively used to assess the mobility and bioavailability of heavy metals in soil and sediment [3, 15, 24, 33, 34]. The distribution of Pb and Cd in soils of the control and the nHA-treated was shown in Fig. 3. Initially, Pb was primarily associated with the CB (65.7%) while Cd was mainly of CB (49.7%) and RS (32.7%), respectively.

With the increase of addition molar ratios of nHA, the EX, CB and RS changes greatly. The EX decreased from 5.5% (control) to 2.5% (5:1) for Pb and from 32.7% (control) to 18% (5:1) for Cd. The CB decreased from 65.7% (control) to 43.9% (5:1) for Pb and from 49.7% (control) to 39% (5:1) for Cd, while the RS was increased to 27% for Pb and increased to 8.3% for Cd respectively. In addition, the nHA decreased OX Pb by about 2.3% and Cd by 1.5% across the treatment; the OM also decreased by about 3.5% (4:1) for Pb, while no obvious increase or decrease for Cd. This result reflected that nHA is less effective for immobilization of Cd than Pb. Though different fractional contents between previous studies and ours have been observed, the decline of EX and OB fractions and the increase of RS fractions were demonstrated after nHA exert. This result also indicated that the nHA-induced can effect immobilize heavy metals (Pb/Cd) in contaminated soil and reduced the bioavailable Pb/Cd to an acceptable level, thus reducing their environment risk.

3.3. Pb/Cd extractability and bioavailability

Metal mobility was closely associated with their water solubility; and metals with higher water solubility would pose higher risk of leaching into water, then threat the organism's health. As Table. 4 showed, the application of nHA had significantly reduced water soluble Pb with 72% reduction and Cd with 90% reduction in contaminated soil. Reduction of water soluble Pb and Cd in the nHA treatment was attributed to transformation of Pb/Cd from soluble Pb/Cd to insoluble.

Bioaccessible Pb/Cd in soil was determined using the PBET method (Table. 4). In the control soil, 52.4% of Pb and 69.33% of Cd on average were bioaccessible, respectively. The nHA significantly reduced bioaccessible Pb with a reduction of 12.5-27.5% and Cd with a reduction of 17.66-34.64%, respectively. Reduction of bioaccessible Pb/Cd may be attributed to high acidity (pH=2) in the PBET solution which would increase further dissolution of soil heavy metals and nHA to lead more calcium lead phosphate hydroxide formation.

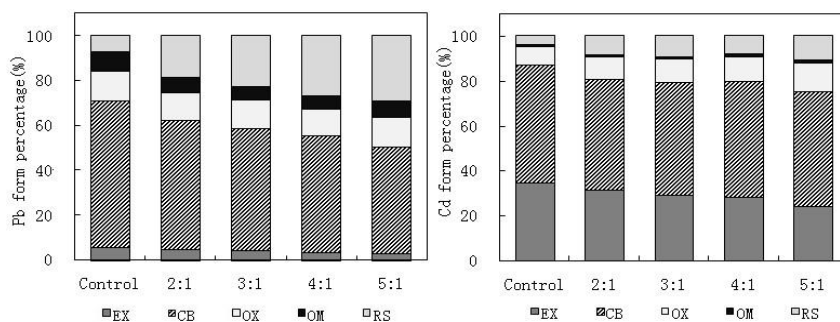


Fig. 3. Percentage of Pb and Cd in each fraction after different levels of nHA.

Table 4. Total DW-extractable heavy metal (mg/kg soil) and PBET with nHA addition.

	Total		DW-extractable		PBET (%)	
	Pb	Cd	Pb	Cd	Pb	Cd
Control	806±16.4	42.7±1.2	2.75±0.17	1.07±0.01	52.40	69.33
2: 1	817±15.6	38.6±0.6	1.86±0.19	0.49±0.02	39.95	51.67
3: 1	838±9.8	41.3±1.1	1.35±0.14	0.34±0.01	29.63	40.48
4: 1	824±15.3	40.8±1.5	0.96±0.16	0.24±0.02	26.92	35.52
5: 1	797±18.5	39.8±3.1	0.78±0.09	0.22±0.03	24.92	34.69

3.4. Pb/Cd phytoavailability

At the end of the experiment, average concentrations of Pb and Cd in shoots of corns grown in the untreated soil (control) were 23.6 (±2.4, n=3) mg kg⁻¹ DW and 14.4 (±2.7, n=3) mg kg⁻¹ DW (Fig. 4). Compared with the control, concentrations of Pb and Cd in shoots of corns were reduced with the addition of nHA. Pb and Cd concentration in shoots were lowered by 65.3% and 64.6%, respectively for molar ratios of P/(Pb+Cd) at 5:1. Plant available Pb and Cd were highly correlated with water soluble Pb and Cd (r=0.913 for Pb; r=0.952 for Cd; P<0.05). Therefore, we speculated that reduction of Pb/Cd water solubility may directly result the reduction of metal phytoavailability. Other studies have shown that metal phytoavailability is positively related to metal concentrations in soil solution [2, 11, 35]. In similar studies, reduced Pb concentration in St. Augustine grass and sudax were observed in contaminated soils with phosphate induced [15, 36]. Furthermore, following nHA added, P nutrient was elevated which would promote plant growth, increasing the biomass.

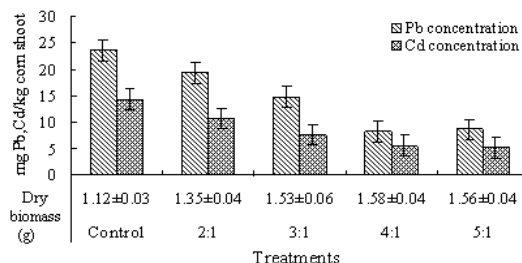


Fig. 4. Average concentration of Pb and Cd in shoots of corn.

4. Conclusions

This study investigated the effectiveness of different amount of nHA in immobilizing Pb, Cd in contaminated soils. The nHA treatment reduced Pb/Cd availability in this study, showing a significant reduction of water soluble, bioaccessible and phytoavailable Pb/Cd by forming the Pb/Cd phosphate (e.g., hydroxypyromorphite-like mineral). The immobilization mechanism main involves both cation exchange and partial dissolution of the nHA amendment and precipitation of heavy metal-containing phosphates. Furthermore, treatment efficiency increased as the nHA dose (based on the molar ratio of P/(Pb+Cd)) increased, and it was observed that immobilization was the most effective when using the molar ratio of P/(Pb+Cd) at 5:1. The results in this study showed that nHA can immobilize Pb and Cd in contaminated soil effectively.

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