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Original Research

Effects of Combined Amendments on Growth and Heavy Metal Uptake by Pakchoi (*Brassica chinensis* L.) Planted in Contaminated Soil

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Abstract

Remediating soils with heavy metals contamination is a significant challenge. This study investigated the co-remediation effect when paired soil amendment mixtures were used to treat soil contaminated by Pb, Cd, and Zn. The three mixed amendments are:

1) micro particle hydroxyapatite (mHAP) and humic acid
2) nano particle hydroxyapatite (nHAP) and humic acid
3) sepiolite and humic acid.

Co-remediation with these amendment pairs was compared with remediation using mHAP, nHAP, sepiolite, and humic acid on their own. Using pot experiments, the Pb, Cd, and Zn fractions of sequential extraction in the contaminated soil and the concentration of Pb, Cd, and Zn in shoots and roots of pakchoi were measured by the use of inductively coupled plasma optical emission spectrometry (ICP-OES). Mixed and single treatments were applied to investigate the differences in remediation effects for the three metals. Study results showed that the co-application of amendments more effectively reduced Pb, Cd, and Zn in pakchoi shoots and roots than with single amendments alone. Co-remediation with the amendment mixtures reduced non-residual fractions of Pb, Cd, and Zn in the contaminated soil more than single amendment supplements. Furthermore, the co-application of 30 g·kg^{-1} micro particle hydroxyapatite with 20 g·kg^{-1} humic acid was most effective in reducing the amount of Pb, Cd, and Zn absorbed by the pakchoi and the non-residual fraction of Pb, Cd, and Zn in the contaminated soil. Results suggest that co-remediation using 30 g·kg^{-1} micro particle hydroxyapatite with 20 g·kg^{-1} humic acid may be an efficient method to remediate Pb, Cd, and Zn in heavy metals-contaminated soil.

Keywords: heavy metal, soil contamination, nano particle hydroxyapatite, sepiolite, humic acid

Introduction

Many researchers have engaged in studies about soils contaminated with heavy metals, because of the threats posed to ecosystems and human health. Many remediation methods – such as phytoremediation, immobilization, chemical washing, and mechanical separation – have been successfully applied to remediate this type of contamination [1-3]. Applying soil amendments to immobilize heavy metals is a promising technology to meet the requirements for environmentally friendly, convenient, and cost-effective remediation operations [4, 5]. Supplemental soil amend-

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Table 1. Basic soil properties.

pH	Organic matter ($g \cdot kg^{-1}$)	Available ($mg \cdot kg^{-1}$)			Total ($mg \cdot kg^{-1}$)		
		N	P	K	Pb	Cd	Zn
6.48	30.7	43.9	32.3	69.0	1,139.2	7.54	321.9

ments reduce the solubility and bioavailability of heavy metals in contaminated soil, protecting plants in that environment. Therefore, identifying cost-effective amendments to remediate heavy metals in contaminated soil is of increasing importance.

Many natural materials such as limestone, zeolite, hydroxyapatite, sepiolite, palyorskite, and red mud have been reported to effectively remediate soil contaminated with heavy metals [6-10]. Among these amendments, hydroxyapatite is considered one of the most effective natural materials in immobilizing heavy metals in soil [11, 12]. With the rapid development of nanotechnology, nanoparticles of hydroxyapatite has been effectively applied to immobilize and remediate toxic heavy metals in soil [13-15]. However, until now little research has been done comparing the effect of nano- and micro-hydroxyapatite (nHAP and mHAP) in remediating heavy metals-contaminated soils. It was hypothesized that nHAP was superior to mHAP in immobilizing heavy metals in soil due to its smaller particle size, larger surface area, and more chemical activity sites [8].

Sepiolite is another chemical soil amendment that has been considered as a useful remediation agent for immobilizing heavy metals in soils [1, 7, 9]. Sun reported that in cadmium (Cd)-contaminated soils, a supplemental application of 10 $g \cdot kg^{-1}$ sepiolite led to a 58.5-65.5% increase in spinach shoot biomass, compared with the control group. Moreover, the Cd concentrations in the spinach shoots and roots decreased by 38.4-59.1% and 12.6-43.6%, respectively, in contrast to the control [7].

The other chemical soil amendment attracting extensive attention is organic matter, which has the ability to transform metals to form stable complexes with organic legends [6, 16]. Humic acids, the most abundant fraction of decomposed organic matter, has been a focus of some research, including studies that have found that humic acids could immobilize toxic heavy metal in soils by redistributing them into less available forms [17, 18].

Although past research has evaluated the effect of natural materials and humic acid on soil contaminated with heavy metals, applying these materials together has not been done previously. Therefore, this study's objectives were to:

1) examine the effects of nHAP and mHAP on the immobilization of Pb, Cd, and Zn in contaminated soil
2) evaluate the effectiveness of nHAP, mHAP, seolite, and humic acid as amendments to co-remediate Pb-, Cd-, and Zn-contaminated soil
3) investigate the effectiveness of different chemical treatments on the uptake of Pb, Cd, and Zn by pakchoi (*Brassica chinensis* L.).

Material and Methods

Soil Sample Properties

The soil samples used for this study (contaminated with Pb, Cd, and Zn) were collected from a suburban area in Shantou City, Guangdong Province, China. The heavy metals in the soil are assumed to have come from local smelting industries. After being air-dried, the soil samples were ground and passed through a 2 mm sieve for pot experiments. Soil pH (soil-to-water ratio of 1:2.5) was determined using a combination pH electrode. Soil organic matter was measured using wet digestion with $K_2Cr_2O_7$ and H_2SO_4. Total Pb, Cd, and Zn in the soil was determined using inductively coupled plasma optical emission spectrometry (ICP-OES), following HF-HNO_3-$HClO_4$ digestion procedures [19]. Available N, P, and K were analyzed using standard methods recommended by Lu [19]. Table 1 provides the soil chemical characteristics and Pb, Cd, and Zn concentrations.

Pot Experimental Design

The pot experiment was conducted using 15 treatments, with four replicates per treatment level (Table 2). Soil samples (3.0 kg each) were placed into individual plastic pots. Before adding treatments, 6.0 $g \cdot pot^{-1}$ soil as NH_4NO_3 and 6.0 $g \cdot pot^{-1}$ soil potassium were added as fertilizers. Soil amendments were thoroughly mixed with soil samples after being air-dried and passed through a 60-mesh sieve. Amendments included nano particle hydroxyapatite (nHAP), micro particle hydroxyapatite (mHAP), sepiolite, and humic acid. The nHAP and mHAP were bought from the Emperor Company in China, and the sepiolite and humic acid were bought from Guangzhou Chemical Reagent Company in China. Deionized water was used to maintain 20% of the soil water-holding capacity. The soil was left to equilibrate for 30 days before planting the pakchoi (*Brassia chinensis* L.). Ten pre-germinated seeds were sown in each pot. Seven days after seedlings emerged, the seedlings were thinned to six per pot. The plants were then grown in a greenhouse at temperatures between 25 and 30ºC. The pakchoi was harvested two months after seed germination.

Metal Analysis

After harvesting, plant roots were carefully removed from each pot, and the shoots and roots were washed using deionized water to wash away adhering soil particles. The shoots and roots were then placed in an oven to dry at

Table 2. Pot experiment treatments.

Treatment	Material added	Amount (g/kg)
Control	Only soil and fertilizer	-
H1	Hydroxyapatite	15
H2	Hydroxyapatite	30
H3	Hydroxyapatite and humic acid	15 hydroxyapatite +10 humic acid
H4	Hydroxyapatite and humic acid	30 hydroxyapatite +20 humic acid
N1	Nano particle Hydroxyapatite	15
N2	Nano particle Hydroxyapatite	30
N3	Nano particle Hydroxyapatite and humic acid	15 Nano particle Hydroxyapatite +10 humic acid
N4	Nano particle Hydroxyapatite and humic acid	30 Nano particle Hydroxyapatite +20 humic acid
S1	Sepiolite	15
S2	Sepiolite	30
S3	Sepiolite and humic acid	15 Sepiolite +10 humic acid
S4	Sepiolite and humic acid	30 Sepiolite +20 humic acid
HA1	Humic acid	10
HA2	Humic acid	20

60ºC for 72 hours. Then the pakchoi were cut into shoot and root parts, and their dry weights were recorded. To measure Pb, Cd, and Zn concentrations in pakchoi tissues, plant samples were digested in a 15 ml HNO_3 and $HClO_4$ solution until the color became clear. At that point, the samples were filtered, reconstituted to the desired volume, and then tested using ICP-OES for Pb, Cd, and Zn concentrations.

pH Determination and Sequential Extraction of Soil Samples

After plants were harvested, a composite soil sample was taken from each pot. Soil pH was determined with a soil-to-deionized-water ratio of 1:2.5 (w/v) using a combination pH electrodes.

A sequential extraction procedure developed by BCR (European Community Bureau of Reference) was used in this experiment [20]. Each chemical fraction was operationally defined as follows:

1. Acid-soluble fraction: 40 ml 0.1 $mol \cdot L^{-1}$ HOAc was added to a 100-mL centrifuge tube containing 1.000 g dried soil. The tube was shaken for 16 h using a mechanical shaker. After equilibration, the supernatant was centrifuged at 4,000 rpm for 10 min. The supernatant was filtered through a 0.45 μm filter, transferred into a polyethylene container, and stored at 4ºC until analysis.
2. Reducible fraction: The residue from the acid-soluble fraction was extracted with 40 mL 0.5 $mol \cdot L^{-1}$ NH_2OH-HCl (pH of 1.5). The tube was shaken for 16 h, and the extraction procedure from step 1 was performed.
3. Oxidizable fraction: The residue from the reducible fraction was extracted with 10 mL H_2O_2 for 1 h at 85ºC, with an additional 10 mL H_2O_2 for 1 h at 85°C. Then, 50 mL 1 $mol \cdot L^{-1}$ NH_4Ac was added, the sample was shaken for 16 h, and the extraction procedure from step 1 was performed.
4. Residual fraction: The residue from the oxidizable fraction was digested with mixed acid composed of HNO_3-$HClO_4$-HF.

After each extraction, separation was performed using a centrifuge at 10,000 rpm for 20 min. Metal concentrations in the soil solutions were determined using ICP-OES.

Statistical Analysis

Microsoft Excel 2003 was used to calculate means and standard deviations (SD). Statistical analysis, including the analysis of variance, was conducted using SPSS Version 17.0 software (SPSS Inc., USA). Differences between means were determined using the Duncan test, with a significance level of $p<0.05$. Figures were plotted using Origin 7.5.

Results and Discussion

Effects of Different Treatments on Soil pH

Fig. 1 shows the effects of chemical treatments on soil pH. Soil pH increased with increased treatment levels. At lower treatment levels (H1, H3, N1, N3, S1, S3, and HA1), pH was incrementally higher than the control by 0.22 to

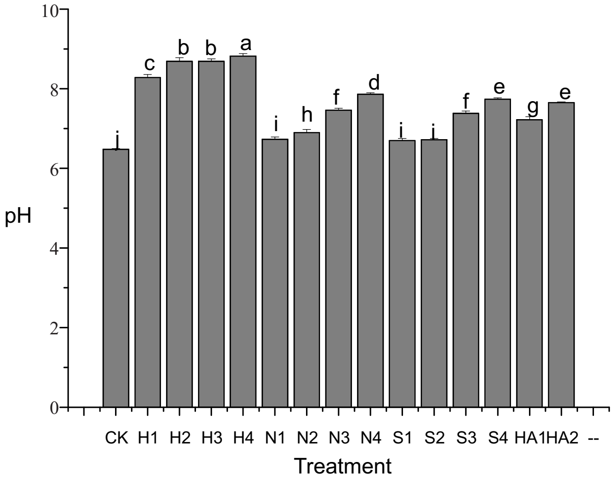

Fig. 1. Effects of different treatments on soil pH. Bars with the same letter are not significantly different, P<0.05.

2.21. At higher treatment levels (H2, H4, N2, N4, S2, S4 and HA2), pH was incrementally higher than the control by 0.24 to 2.34. Applying both natural minerals and humic acid-enhanced soil pH more than that when using a single amendment. For example, when using natural minerals without humic acid (H1, H2, N1, N2, S1 and S2), the soil pH increased by 0.22 to 2.21 units compared to the control. After supplementing natural minerals with humic acid (H3, H4, N3, N4, S3, and S4), soil pH increased by 0.90 to 2.34.

These results confirmed that natural minerals co-applied with humic acid showed more efficiency in increasing soil pH. Fig. 1 shows that the H4 treatment level increased soil pH the most among all amendments, increasing soil pH by 2.34 units compared to the control. This result corresponds with the results of Cui [8], who reported that supplemental hydroxyapatite can enhance soil pH, and that the incremental increase is consistent with the amount of hydroxyapatite added. Treatment N4 also experienced an increase in soil pH, by 1.38 units. Due to the alkalinity properties of sepiolite [1], Treatment S4 experienced a soil pH increase of 1.26 units. For treatment HA2, the soil pH was increased by 1.17 units.

Sequential Extraction of Pb, Cd, and Zn in Soils

BCR is one of the sequential extraction schemes established by the European Community Bureau of Reference, and is a standard method for comparing and evaluating metals in soil. Using this method, extracted metal concentrations in contaminated soil samples can be compared and evaluated using the sequential extraction of different forms of metal in soil. This includes the acid-soluble fraction (weakly bound with organic matter and carbonate fraction), reducible fraction (iron and manganese oxides fraction), oxidizable fraction (organically bound and sulfide fraction), and residual fraction [21]. Non-residual metals, which consist of the acid-soluble fraction, reducible fraction, and oxidizable fraction, are more mobile and bioavailable than the residual fraction. As such, the effectiveness of *in situ* remediation of metals-contaminated soils can be calculated using a fraction scheme. The more effective amendments transform greater amounts of metal from the non-residual to the residual fraction.

Fig. 2a shows the proportions of four fractions of Pb. In untreated soil, most of the Pb was strongly associated with the reducible fraction, followed by the residual fraction, which was larger than the acid-soluble fraction. All the amendments were able to partition Pb from the non-residual fraction to the residual fraction, indicating that all amendments consistently decreased Pb mobility and bioavailability. The mHAP and nHAP transferred most of the Pb's reducible fraction, acid-soluble fraction, and oxida-

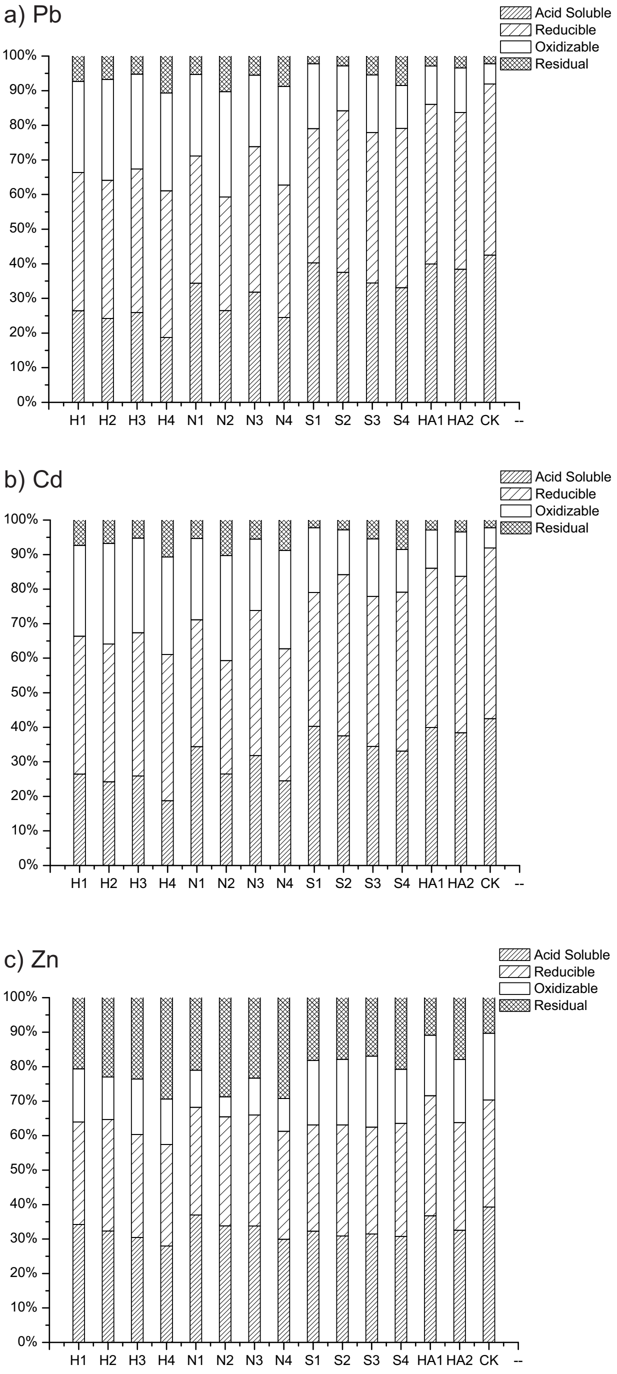

Fig. 2. Relative percentages of Pb, Cd, and Zn fractions in soils from CK and with various amendment treatments.

Table 3. Concentrations of Pb, Cd, and Zn in pakchoi shoots and roots with different treatments (mg·kg^{-1} DW).

Treatment	Pb in shoots	Pb in roots	Cd in shoots	Cd in roots	Zn in shoots	Zn in roots
Control	16.43 ± 2.54^{a}	58.52 ± 4.06^{a}	4.17 ± 0.30^{a}	9.63 ± 1.14^{a}	23.39 ± 2.69^{a}	58.24 ± 5.06^{a}
H1	6.26 ± 0.36^{def}	19.66 ± 3.26^{ef}	1.65 ± 0.18^{def}	3.00 ± 0.41^{h}	16.69 ± 3.67^{dfg}	26.96 ± 4.08^{fg}
H2	5.40 ± 1.04^{ef}	14.13 ± 0.82^{ghi}	1.36 ± 0.20^{f}	2.25 ± 0.16^{i}	14.40 ± 2.53^{ghi}	18.47 ± 1.71^{i}
H3	5.14 ± 0.63^{fg}	15.89 ± 2.71^{gh}	1.43 ± 0.19^{ef}	2.67 ± 0.24^{hi}	15.68 ± 1.88^{dfgh}	21.61 ± 2.86^{ghi}
H4	4.50 ± 0.39^{f}	13.09 ± 1.69^{i}	0.94 ± 0.22^{g}	2.06 ± 0.25^{i}	11.05 ± 1.63^{i}	16.33 ± 1.80^{i}
N1	6.59 ± 1.07^{ef}	19.11 ± 1.63^{ef}	2.25 ± 0.16^{c}	4.27 ± 0.21^{ef}	18.11 ± 2.54^{cdfg}	36.11 ± 2.53^{cd}
N2	5.84 ± 0.82^{ef}	16.67 ± 2.45^{gh}	1.68 ± 0.19^{def}	3.83 ± 0.16^{fg}	15.09 ± 0.81^{fgh}	24.37 ± 1.82^{gh}
N3	5.71 ± 1.22^{ef}	17.83 ± 2.29^{fg}	1.75 ± 0.16^{de}	3.78 ± 0.15^{fg}	17.87 ± 2.12^{cdfg}	30.14 ± 2.56^{ef}
N4	5.56 ± 0.41^{ef}	14.46 ± 1.71^{hi}	1.34 ± 0.11^{f}	3.28 ± 0.33^{gh}	12.57 ± 1.96^{hi}	21.32 ± 3.43^{hi}
S1	11.09 ± 1.63^{b}	30.87 ± 3.51^{cd}	1.98 ± 0.08^{cd}	5.37 ± 0.89^{cd}	19.61 ± 2.62^{bcd}	46.53 ± 5.88^{b}
S2	10.13 ± 0.10^{bc}	22.95 ± 1.95^{e}	1.83 ± 0.19^{d}	5.13 ± 0.43^{d}	18.57 ± 2.68^{bcdf}	36.51 ± 2.77^{cd}
S3	8.71 ± 2.04^{cd}	27.64 ± 2.69^{d}	1.68 ± 0.34^{def}	4.73 ± 0.29^{de}	18.32 ± 1.88^{cdfg}	40.01 ± 4.08^{c}
S4	7.76 ± 1.10^{de}	21.44 ± 2.64^{e}	1.44 ± 0.18^{ef}	4.20 ± 0.12^{ef}	16.15 ± 1.76^{dfgh}	33.38 ± 1.80^{de}
HA1	11.85 ± 2.84^{b}	35.71 ± 4.25^{b}	3.87 ± 0.41^{a}	7.01 ± 0.82^{b}	22.32 ± 3.53^{ab}	49.14 ± 5.79^{b}
HA2	10.42 ± 0.98^{bc}	32.88 ± 4.08^{bc}	2.72 ± 0.17^{b}	5.97 ± 0.35^{c}	20.72 ± 2.61^{abc}	46.11 ± 3.35^{b}

Data with a different letter in the same column indicate a statistically significant difference, according to the Duncan test. $P<0.05$.

tive fraction to the residual fraction. Sepiolite and humic acid transformed a substantial amount of the acid-soluble fraction to the residual fraction.

The H4 treatment level showed the greatest effect in converting Pb from the non-residual to the residual fraction. The acid-soluble fraction portion decreased from 14.89% in the control to 1.91% in the H4 treatment soil, while the residual fraction increased from 32.67% in the control to 92.55% in the H4 treatment soil. The N4 treatment also reduced the Pb portion of acid-soluble fraction from 14.89% in the control to 2.49% in the treatment; the residual fraction increased from 32.67% to 89.64%. In comparing the ability of H4 and N4 treatments to modify the Pb fraction distribution, the main difference was that a lower proportion of Pb's acid-soluble fraction was found with H4 than with N4. The proportion of Pb's acid-soluble fraction was reduced from 14.89% in the control to 6.40% in the S4 treatment level. Meanwhile, the residual fraction increased from 32.67% to 46.29%. The HA2 treatment decreased Pb's acid-soluble fraction from 14.89% to 8.21%, and increased the residual fraction of Pb from 32.67% to 41.40%.

Fig. 2b shows the proportions of the four fractions of Cd. In the control samples, Cd was predominantly in reducible and acid-soluble forms, indicating that a substantial fraction of Cd may be available for the plant. The residual fraction was the smallest portion of four fractions. All the amendments altered Cd distribution. The amendments mHAP and nHAP reduced a large amount of the reducible and acid-soluble fractions and enhanced the oxidative and residual fractions in contaminated soil. Sepiolite and humic acid slightly decreased the reducible and acid-soluble fractions and raised the amount of oxidative and residual fractions. The H4 treatment greatly decreased acid-soluble Cd and increased the oxidative fraction. This suggests that the HAP mechanism for the Cd reaction may be different from that of Pb. The acid-soluble fraction of Cd was significantly reduced from 42.50% in the control to 18.74% with the H4 treatment, and to 24.51% with the N4 treatment. The S4 treatment changed the Cd distribution from the acid solution fraction to the oxidative and residual fractions. With the S4 treatment, the portion of acid solution fraction was reduced from 42.50% in the control to 33.09%, and the residual fraction increased from 2.21% to 8.54%. Adding HA2 reduced 42.50% of the acid-soluble fraction to 38.43%.

Fig. 2c shows the proportions of the four fractions of Zn. Similarly to Cd, the Zn in the control samples was mainly comprised of reducible and acid-soluble fractions, followed by the oxidative fraction, which was larger than the residual fraction. Amendment treatments altered the partitioning of the Zn fraction. The mHAP and nHAP reduce the proportion of acid solution and oxidative fraction, increase the residual fraction, and slightly decrease the reducible fraction. Sepiolite and humic acid appear to reduce the acid solution fraction and increase the residual fraction. The H4 treatment was the most effective in decreasing the Zn acid solution fraction and increasing the residual fraction. When compared to untreated soil, the treatments resulted in a reduction of the Zn acid-soluble fraction portion in a range from 39.28% to 28.01%; the Zn residual fraction ranged from 10.30% to 29.39% after the H4 treatment.

Table 4. Shoot and root biomass of pakchoi (*Brassia chinensis* L.) with different treatments.

Treatment	Shoot	Root
	(g·pot^{-1} DW)	
Control	1.25 ± 0.06^{i}	0.24 ± 0.01^{h}
H1	4.44 ± 0.39^{cd}	0.64 ± 0.02^{d}
H2	5.60 ± 0.28^{b}	0.99 ± 0.07^{b}
H3	4.65 ± 0.30^{cd}	0.87 ± 0.08^{b}
H4	6.03 ± 0.57^{a}	1.05 ± 0.04^{a}
N1	3.50 ± 0.14^{e}	0.40 ± 0.02^{f}
N2	4.34 ± 0.25^{d}	0.44 ± 0.03^{e}
N3	3.59 ± 0.33^{e}	0.48 ± 0.03^{de}
N4	4.77 ± 0.29^{c}	0.57 ± 0.06^{b}
S1	2.36 ± 0.17^{g}	0.29 ± 0.01^{f}
S2	3.09 ± 0.23^{f}	0.39 ± 0.03^{f}
S3	2.85 ± 0.15^{f}	0.39 ± 0.05^{c}
S4	3.66 ± 0.25^{e}	0.45 ± 0.02^{b}
HA1	1.80 ± 0.29^{h}	0.31 ± 0.02^{f}
HA2	2.18 ± 0.19^{g}	0.36 ± 0.02^{f}

Data with a different letter in the same column indicate a statistically significant difference, according to the Duncan test; $P<0.05$.

In addition, it was found that H1 to H4 treatments were more effective in shifting the non-residual fractions to the residual fractions than other treatment types. This indicates that hydroxyapatite (especially when co-applied with humic acid) is suitable for use with Zn-contaminated soil. N4 and S4 also decreased the acid-soluble fraction and increased the residual fraction, the reductions were 9.33% for N4 and 8.53% for S4 for the acid-soluble fraction, and the increments were 18.91% for N4 and 10.45% for S4 for the residual fraction. This indicates that both N4 and S4 have the ability to transform the higher bio-availability fraction of Zn to a lower bio-availability form, immobilizing Zn in contaminated soil. HA2 translocated the acid-soluble fraction of Zn from 39.28% in the control to 32.53% in the treatment and slightly increased the residual fraction.

In this study, a higher concentrated treatment usually resulted in a significantly lower concentration of non-residual Pb, Cd, and Zn than similar treatments at lower concentrations. When examining the Pb, Cd, and Zn fractions during sequential extraction, the co-remediation effect of mHAP, nHAP, and sepiolite with humic acid were more effective in decreasing Pb, Cd, and Zn mobility and bioavailability in the polluted soil when compared to single amendment remediation. H4 appeared to be the most effective treatment in transforming acid fractions of Pb, Cd, and Zn into the residual fraction, suggesting that H4 was most suitable for treating Pb-, Cd-, and Zn-contaminated soil. This opens new options for researching the co-remediation of heavy metal-contaminated soil, and for applying natural minerals in environmental remediation.

Effects of Different Amendments on the Uptake of Pb, Cd, and Zn by Pakchoi

Table 3 provides Pb, Cd, and Zn concentrations in pakchoi shoots and roots, and show that amendments greatly decreased Pb, Cd, and Zn levels in the plant compared to the control. At the lower treatment levels (H1, H3, N1, N3, S1, S3, and HA1), Pb, Cd, and Zn concentrations in the shoots were reduced by 27.9-68.7% for Pb, by 7.2-65.7% for Cd, and by 4.6-32.9% for Zn. Meanwhile, Pb, Cd, and Zn concentrations in the roots were reduced by 38.9-72.8% for Pb, by 27.2-72.2% for Cd, and by 15.6-62.9% for Zn.

With the higher treatment levels (H2, H4, N2, N4, S2, S4, and HA2), the Pb, Cd, and Zn concentrations in the shoots decreased by 36.6-72.6% for Pb, 34.8-77.4% for Cd, and 11.4-52.8% for Zn. In the meantime, Pb, Cd, and Zn concentrations in the roots were reduced by 43.8-77.6% for Pb, 38.0-78.6% for Cd, and 20.8-71.9% for Zn, compared to the control. These results indicate that increasing amendment application rates may restrain the amount of Pb, Cd, and Zn used by pakchoi.

Co-applying natural minerals with humic acid reduced more Pb, Cd, and Zn in pakchoi than with one amendment alone. When treated with natural minerals with humic acid (H3, H4, N3, N4, S3, and S4), the Pb, Cd, and Zn in pakchoi shoots were inhibited by 46.9-72.6% for Pb, 59.7-77.4% for Cd, and 21.6-52.8% for Zn. For roots, the reductions were 52.8-77.6% for Pb, 50.9-78.6% for Cd, and 31.3-71.9% for Zn, in contrast to the control. When the amendment was used alone (H1, H2, N1, N2, S1, and S2), Pb, Cd, and Zn in pakchoi shoots were 32.4-67.1%, 46.0-67.3%, and 16.1-38.4%, respectively. The reductions in roots for the three metals were 47.2-75.9%, 44.2-76.6%, and 20.1-68.3%, respectively, compared to that of control.

The H4 treatment was the most efficient at reducing Pb, Cd, and Zn uptake by the plant. After applying H4, Pb, Cd, and Zn concentrations decreased by 72.6%, 77.5%, and 52.8% for shoots, and 77.6%, 78.6%, and 72.0% for roots. Pb, Cd, and Zn in shoots and roots decreased significantly when the N4 treatment was added. Pb, Cd, and Zn was reduced by 66.1%, 67.9%, and 46.3% for shoots, and 75.3%, 65.9%, and 63.4% for roots, compared with untreated soil. Amending soils with S4 led to a significant reduction in Pb, Cd, and Zn uptake into pakchoi shoots and roots: 52.8% for Pb, 65.5% for Cd, and 30.9% for Zn decrease in shoots; 63.4% for Pb, 56.4% for Cd, and 42.7% for Zn decrease in roots, when compared to control. HA2 was less effective than the treatments discussed above, reducing Pb, Cd, and Zn in the pakchoi shoots by 36.6%, 34.8%, and 11.4%, and by 43.8%, 38.0%, and 20.8%, respectively, in the roots.

Effects of Amendments on Pakchoi Biomass

The inhibitory effects on amendments on plant biomass provide an index of phytotoxicity; a biomass increase as a result of amendment application could predict a toxicity reduction [22]. Results in Table 4 demonstrate that all amendments significantly increased pakchoi shoot and root biomass. Furthermore, shoot and root biomass consistently increased with an increased treatment level. At lower treatment levels (H1, H3, N1, N3, S1, S3, and HA1), shoot yield was 0.43 to 2.71 times the control's yields; root yields were 0.21-2.63 times the control. At higher treatment levels (H2, H4, N2, N4, S2, S4, and HA2), shoot yield enhancement was 0.74-3.81 times the control, and root yields were 0.50-3.38 times the control.

Further, compared to the single amendment treatment, the co-application of the natural minerals mixed with humic acid enhanced shoot and root biomass. For example, after applying natural minerals with humic acid (H3, H4, N3, N4, S3, and S4), shoot biomass increased by 1.27-3.81-fold, and root biomass increased by 0.63-3.38-fold, compared with the control. When the natural minerals were applied without humic acid (H1, H2, N1, N2, S1, and S2), shoot biomass incremental growth was by 0.88-3.47-fold and the root biomass growth was 0.21-3.13-fold. These results demonstrate that co-applying natural minerals with humic acid was more efficient than a single natural minerals treatment in increasing pakchoi biomass.

In this study, the lower pakchoi biomass in the control may be due to low soil pH and high metal toxicity. Chemical amendments can promote plant growth by increasing soil pH; decreasing the bioavailable fraction of Pb, Cd, and Zn; and enhancing pakchoi biomass. In addition, as an organic fertilizer, humic acid offers nutrition for the pakchoi and boosts shoot and root development [17]. Among all the amendments, the H4 treatment level exhibited the best effect in increasing pakchoi shoot and root biomass. When treated with H4, pakchoi shoot and root biomass increased by 3.81 and 3.38 times, respectively, compared with the control. The N4 treatment level enhanced pakchoi shoot and root biomass by 2.81 and 1.38 times, compared with untreated soil. Further supplementing the plant with S4 promotes pakchoi growth by increasing the yield by 1.92 times for the shoot and 0.88 times for the root. Applying HA2, the increment of shoots and roots yield was 74.0% and 50.0%, compared to the control. Among the four treatments (H4, N4, S4, and HA2), the H4 treatment showed the best effect in increasing plant shoot and root yield, followed by N4. The HA2 treatment has the least effect on plant growth. These results are consistent with the decreased Pb, Cd, and Zn concentrations in plant shoots and roots, and confirmed that the amendments both alleviated heavy metal phytotoxicity and benefitted pakchoi growth.

Precipitates such as metal carbonates, hydroxides and metal-organic complexes, are more likely to form at high pH conditions [23]. The increase in soil pH results in more negatively charged sorption sites on the soil colloid and organic matter surfaces, decreasing metal bio-availability [24, 25]. In this study, the supplemental hydroxyapatite treatment can increase soil pH and reduce metal bioavailability. Bolisson [26] demonstrated the mechanism by which hydroxyapatite increases soil pH, finding that the dissolution of hydroxyapatite in soil can consume H^+. The reaction is as follows:

$$Ca_{10}(PO_4)_6(OH)_2+14H^+=10Ca^{2+}+6H_2PO_4^-+2H_2O$$

Furthermore, hydroxyapatite can react with F^- in soil to form $Ca_{10}(PO_4)_6F_2$, which is insoluble in the soil. As a result, more H^+ can be consumed and soil pH increases [27]. In this study, mHAP increased soil pH values more effectively than nHAP. This may be due to the fact that nanomaterials can easily aggregate, changing their surface sorption and migration properties, and decreasing their dissolution rates [28]. Micrometer HAP has larger particles than nanometer HAP, and is therefore easier to dissolve in the soil. As such, the larger dissolution rate of mHAP may drive the increased pH phenomenon [8]. Results also showed that adding sepiolite can increase pH in contaminated soil. This is because sepiolite contains a high percentage of calcium carbonate, giving it alkaline properties [1, 9].

There are several reasons explaining the hydroxyapatite remediation effects on Pb-contaminated soil. Adsorption, desorption, precipitation, rhizosphere modification, and physiological reactions reduce Pb mobility and bioavailability. However, a key Pb immobilization process involving hydroxyapatite is the formation of pyromorphite, or other compounds with low solubility. This is associated with the transfer of large amounts of Pb from the non-residual to residual fractions [29, 30]. This study confirmed that hydroxyapatite amendments significantly increased the residual fraction of Pb in soils, consistent with the reduction of Pb concentration in plant shoots and roots. Hydroxyapatite also modified Cd and Zn distribution in soil, increasing residual Cd and Zn while decreasing non-residual Cd and Zn. Cd and Zn immobilization with hydroxyapatite had different mechanisms than with Pb. As Chen [11] noted, the reduction in Cd and Zn acid-soluble fractions with hydroxyapatite can be attributed to sorption to the hydroxyapatite surface, rather than by insoluble compound formation. Furthermore, increased pH values caused by hydroxyapatite in contaminated soils may lead to the formation of $Cd(OH)_2$ and $Zn(OH)_2$. This results in a large amount of $Cd(OH)_2$ and $Zn(OH)_2$ precipitation [2].

Sepiolite is a natural hydrated magnesium silicate mineral, with the ability to reduce heavy metals bioavailability in soils and inhibit trace element absorption in plants. Many researchers have focused on its use [1, 7]. Liang [9] used sepiolite as an amendment to remediate Cd-contaminated paddy soil. Their study showed that applying sepiolite can increase paddy soil pH, while reducing exchangeable Cd concentrations. The main immobilization mechanisms for sepiolite include: precipitation of Cd as carbonates or hydroxides, and surface complexation. Sun [7] investigated the remediation effects of sepiolite on soils artificially polluted with cadmium. Results indicated that adding sepiolite can increase soil pH and decrease the extractable Cd concentration. Moreover, applying sepiolite significantly restricted Cd uptake in spinach shoots grown in Cd-contaminated soil. In this study, sepiolite effectively shifted heavy metal fractions from non-residual to residual fractions, and reduced Pb, Cd, and Zn uptake in pakchoi. This result is consistent with Sun [1] and Liang [9].

However, when compared with hydroxyapatite and sepiolite, hydroxyapatite appears to be more effective than sepiolite in immobilizing Pb, Cd, and Zn, and in inhibiting heavy metal uptake by pakchoi. For instance, compared to the control, when treated with S4, the acid-soluble fraction decreased by 8.49% for Pb, 9.41% for Cd, and 8.35% for Zn. The incremental residual fraction was 13.62%, 6.33%, and 10.45% for Pb, Cd, and Zn, respectively. For hydroxyapatite, H4 treatment led to a reduction of the acid-soluble fraction by 12.98% for Pb, 23.76% for Cd, and 11.27% for Zn. The incremental residual fraction was 59.88%, 8.52% and 19.09% for Pb, Cd, and Zn, respectively. Larger pH values found in hydroxyapatite-treated soil may help explain this phenomenon. The H4 treatment increased pH value by 2.34 units, while the S4 treatment increased it by 1.26 units; the pH value increase was 2.21 and 0.24 units when treated with H2 and S2, respectively. Heavy metals stability was strongly pH-dependent; mobility and solubility decreased with increasing pH. Natural clays are considered to decrease metal mobility and solubility in contaminated soils by changing soil properties, especially pH [31]. Therefore, sepiolite impacted soil pH less than hydroxyapatite, at the same treatment rate. This resulted in less Pb, Cd, and Zn being immobilized.

This study found that adding humic acid can reduce the non-residual fraction of Pb, Cd, and Zn, while increasing the residual fraction of Pb, Cd, and Zn. This suggests that more available metal fractions in soil were changed into non-available fractions, with less taken up and used by pakchoi. This result was similar to findings by Tordoff [18]. The major benefits of humic acid when applied to soil are due to the incrementally increased organic matter content and biological activity. Acting as a nutrient pool, humic acid can improve soil physical properties and nutrient cycling, increase CEC and buffer capacity, and reduce compaction [16, 32]. Additionally, humic acid can decrease heavy metal bioavailability by translocating them from forms available to plants (such as acid-soluble fractions) to oxidizable and residual fractions. Further, adding humic acid to metals-contaminated soils has an improvement effect due to increased surface area and the enhanced number of specific adsorption sites on soil particles [33]. In addition, the reduction of Pb, Cd, and Zn in plants and non-residual fractions in soil with the increased application levels of mHAP, nHAP, and sepiolite after co-remediation with humic acid confirms that humic acid can improve remediation of Pb-, Cd-, and Zn-contaminated soil. In other words, study results prove that humic acid assists mHAP, nHAP, and sepiolite in enhancing and promoting Pb, Cd, and Zn immobilization in soil. Furthermore, the co-application of 30 $g\cdot kg^{-1}$ hydroxyapatite with 20 $g\cdot kg^{-1}$ humic acid showed the best effect on Pb, Cd, and Zn remediation.

Nanomaterials have a higher reactivity and greater adsorption ability, and some studies have suggested that nanomaterials are superior to traditional amendments using common particle sizes for soil remediation [15]. Theoretically, in this study, compared to mHAP, nHAP would immobilize a larger amount of Pb, Cd, and Zn, likely due to its smaller particle size and larger surface area. However, we noted that mHAP was more effective than nHAP in immobilizing Pb, Cd, and Zn. Moreover, soil pH was more greatly enhanced by mHAP. Gilbert [28] and Cui [8] speculated that nanomaterials can easily aggregate, altering their surface sorption and migration ability while decreasing their dissolution rates. Compared to nHAP, mHAP has a larger particle size, making it much more difficult for mHAP to aggregate. Therefore, we hypothesize that the higher dissolution rate was the primary reason for the increased pH and for the increased immobilization effects. Further study is needed to verify this hypothesis.

Conclusion

Co-remediation, using the amendments studied here, increased pakchoi biomass and reduced Pb, Cd, and Zn levels in pakchoi shoots and roots. In addition, soil pH increased and the bioavailable chemical fraction decreased, in alignment with increasing treatment levels. Across all treatments, co-applying 30 $g\cdot kg^{-1}$ hydroxyapatite with 20 $g\cdot kg^{-1}$ humic acid yielded the best results, indicating that these co-remediation amendments can be applied to Pb-, Cd-, and Zn-contaminated soil. Co-remediation using micro particle hydroxyapatite, nano particle hydroxyapatite, and sepiolite with humic acid was more effective in inhibiting Pb, Cd, and Zn uptake in pakchoi and restraining Pb, Cd, and Zn availability in polluted soil, compared with remediation using a single amendment. Moreover, mHAP was superior to nHAP in immobilizing Pb, Cd, and Zn in metal-contaminated soil and reducing the Pb, Cd, and Zn by pakchoi. In conclusion, our study found that co-remediation using hydroxyapatite with humic acid may effectively treat Pb-, Cd-, and Zn-contaminated soil, offering an improved approach for soil remediation.

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