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RESEARCH ARTICLE

Environmental materials for remediation of soils contaminated with lead and cadmium using maize (*Zea mays* L.) growth as a bioindicator

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Abstract Heavy metal pollution is a severe environmental problem. Remediation of contaminated soils can be accomplished using environmental materials that are low cost and environmentally friendly. We evaluated the individual and combination effects of humic acid (HA), super absorbent polymer (SAP), zeolite (ZE), and fly ash composites (FC) on immobilization of lead (Pb) and cadmium (Cd) in contaminated soils. We also investigated long-term practical approaches for remediation of heavy metal pollution in soil. The biochemical and morphological properties of maize (*Zea mays* L.) were selected as biomarkers to assess the effects of environmental materials on heavy metal immobilization. The results showed that addition of test materials to soil effectively reduced heavy metal accumulation in maize foliage, improving chlorophyll levels, plant growth, and antioxidant enzyme activity. The test materials reduced heavy metal injury to maize throughout the growth period. A synergistic effect from combinations of different materials on immobilization of Pb and Cd was determined based on the reduction of morphological and biochemical injuries to maize. The combination of zeolite and humic acid was especially effective. Treatment with a combination of HA+SAP+ZE+FC was superior for remediation of soils contaminated with high levels of Pb and Cd.

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Keywords Environmental materials · Heavy metal · Lead · Cadmium · Injuries · Immobilization · Soil

Abbreviations

HA	Humic acid
SAP	Super absorbent polymer
ZE	Zeolite
FC	Fly ash composite
Pb	Lead
Cd	Cadmium
OM	Organic matter
PAANa	Sodium polyacrylate
EC	Electrical conductivity
ROS	Reactive oxygen species
MDA	Malondialdehyde
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase

Introduction

Industrial activities have produced serious soil heavy metal pollution worldwide. Heavy metals enter the soil in various ways. The main anthropogenic sources include phosphate fertilizers, sewage sludge, pesticides, and emissions from power plants, metal and cement factories, and vehicles (Kumpiene et al. 2008; Bolan et al. 2003).

Heavy metals are non-degradable and persistent in the soil (Hu and Cheng 2013; Kirkham 2006). They are toxic to both plants and humans and have significant soil mobility (Ahmad et al. 2012). Lead (Pb) and cadmium (Cd) are two widespread heavy metals that can accumulate in soil (Bian et al. 2014; Barrutia et al. 2010). Plants simultaneously exposed to Pb and Cd suffer morphological and physiological injury. Heavy metals adversely affect plant absorption and transport of essential elements, chlorophyll synthesis, growth, and reproduction. Antioxidant enzymes can help alleviate heavy metal toxicity by direct protective mechanisms, but these enzymes are also adversely effected by high concentrations of heavy metals (Gill and Tuteja 2010; Gomes et al. 2006).

Several strategies have been proposed for remediation of soils contaminated with Pb and Cd. These include physical remediation, chemical remediation, and bioremediation (Almaroai et al. 2014; Lim et al. 2013; Silva and Roldan 2009). Application of non-toxic soil additives can be an environmentally safe and effective way to remediate heavy metal pollution. Environmental materials are non-toxic materials which possess properties with minimal environmental impact (Halada and Yamamoto 2001). They have the capacity to immobilize heavy metals in soils by means of their unique functional groups (Huang et al. 2012; Llera et al. 2004). Environmental materials can improve the physicochemical properties of soil and reduce detrimental conditions affecting plant growth (Ok et al. 2011; Oste et al. 2002). Examples of non-toxic soil additives include red mud, kaolinite, zeolite, EDTA, and biochar (González et al. 2014; Jiang et al. 2012; Gray et al. 2006). These materials have been used in the remediation of heavy metal-contaminated soils.

Previous studies mainly focused on the immobilization of heavy metals using a single material for a short time period. The possible synergistic effect of environmental materials on immobilization of Pb and Cd in soils over longer time periods has received less attention. In this study, maize (*Zea mays* L.) was selected as a model plant because of easy cultivation, fast growth, and high biomass production (Fu et al. 2014). Humic acid (HA), super absorbent polymer (SAP), zeolite (ZE), and fly ash composites (FC) were added to contaminated soils as amendments to investigate potential synergistic effects on reducing Pb and Cd accumulation and alleviating biochemical and morphological injuries to maize throughout its growth. Our study suggests new, useful, and practical methods for remediation of soils polluted by heavy metals.

Materials and methods

Soil samples and test materials

Soil samples were composed of silty clay loam (0–20-cm depth). They were collected from suburban farmland in Tongzhou District (N 39° 54′, E 116° 39′) of Beijing, China. Soil samples were air-dried and passed through a 2-mm sieve. The particle size distribution was determined using the pipette method (Gee and Bauder 1986). Soil pH and electrical conductivity (EC) were measured in 1:5 soil-water suspensions using a pH meter (PHS-3C, China) and conductivity meter (DDS-11A, China), respectively. The total concentration soil organic matter (OM) was determined by the Walkley-Black method (Nelson and Sommers 1982). The initial levels of Pb and Cd in soil sample were determined by ICP-MS (Agilent 7700, USA). Physicochemical properties of the soil are shown in Table 1. The environmental background values of Pb and Cd, 19.110 and 0.063 mg/kg, respectively, were negligible compared with the experimental added levels of Pb and Cd.

Maize (*Z. mays* L.) seeds (Nongda 86) were obtained from the Chinese Academy of Agricultural Sciences, Beijing, China. Humic acid (HA) was provided by Neimenggu Huolin Coal Industry Group Co., LTD, China. Super absorbent polymer (SAP) (size 100-mesh polyacrylic acid salt) was obtained from Tangshan Boya Tech Group Co., LTD, China. Zeolite (ZE) (main component is clinoptilolite, size 100 mesh) was obtained from Henan Xinyang Huaiye Coal Mine Group Co., LTD, China. Fly ash composites (FC) (91 % silt and 9 % clay, pH 8.36) were taken from Datong Coal Mine Group Co., LTD, China.

Experimental design

Based on different mechanisms of immobilizing heavy metals, four different test materials were evaluated to deactivate heavy metals lead (Pb) and cadmium (Cd) in soil and inhibit the migration of Pb and Cd to maize (*Z. mays* L.). The experimental treatments were composed of single test material treatments, combination treatments, and two controls. Each treatment had three repetitions. All experimental treatments are summarized in Table 2. In order to enhance the water retention capacity of soil, and simultaneously evaluate the effectiveness of SAP on immobilizing heavy metals, SAP was applied to each combination treatment. The study focused on the potential effect of the combination of test materials on the deactivation and immobilization of Pb and Cd by analysis of the biochemical and morphological properties in maize. Capital letters B, C, and D represent combinations of two, three, and four materials, respectively.

The test material dosages are shown in Table 3. The cost of amendments should always be considered. In this experiment, the doses of different additives were based on their cost and properties, as well as the previous studies in our laboratory. In

Table 1 Selected physicochemical properties of the investigated soil

Clay (%)	Silt (%)	Sand (%)	EC (ds/m)	OM (g/kg)	pH	Total Pb (mg/kg)	Total Cd (mg/kg)
16.23	59.27	24.5	0.28	5.7	7.50	19.11	0.063

[a] Electrical conductivity

[b] Organic matter

Table 2 Composition and design of experimental treatments

Treatment	Test material			
	HA (w/g kg^{-1})	SAP (w/g kg^{-1})	ZE (w/g kg^{-1})	FC (w/g kg^{-1})
HA	√			
SAP		√		
ZE			√	
FC				√
B1	√	√		
B2		√	√	
B3		√		√
C1	√	√	√	
C2	√	√		√
C3		√	√	√
D	√	√	√	√
CK1[a]				
CK2[b]				

[a] Control group: treatment added Pb and Cd in soils without environmental materials

[b] Blank group: treatment without Pb-Cd and environmental materials

addition, one goal of this work was to simulate a condition of the critical value of normal plant growth and agricultural production. According to Chinese environmental quality standard (GB15618-1995) (Liu 2001), the combination of Pb and Cd was added to the soil and the concentrations of Pb and Cd were 500 and 10 mg/kg, respectively.

Pot experiments

A pot experiment was performed at the China University of Mining and Technology—Beijing, China (N 39° 59′, E 116° 20′). The local climate is temperate monsoonal according to Koppen classification. The experiment was conducted in a controlled environment chamber under the night/day temperatures of 20/25 °C. The average relative humidity was 58 % from March 15 to July 16. Pots were 25 cm diam.×30 cm deep, with a drainage hole at the bottom. Each pot was filled with 8 kg of air-dried soil with three replications.

According to the experimental design, the soil, test materials, and heavy metals were mixed thoroughly in pots and wetted to maximum soil water holding capacity. The test soils were then aged at room temperature for 30 days (March 15 to April 14). During the aging time, pots were weighed every 3 days and water was added to maintain a constant moisture content.

After 30-day soil aging, maize seeds were cleaned, disinfected, and immersed in tap water for 24 h. Six hydrated seeds were planted into each pot (two seeds per cave and three caves per pot). Seedlings were thinned to three plants per pot after grown. During growth (April 15 to July 16), the treatments were weighed every 2 days and water was added to 90 % of the soil water holding capacity.

Table 3 Additive dosage of different environmental materials in treatments

	Test materials			
	HA[a]	SAP[b]	ZE[c]	FC[d]
Additive dosage (g/kg)	0.25	2	10	50

[a] Humic acid

[b] Super absorbent polymer

[c] Zeolite

[d] Fly ash composite

Experimental indicator measurement

The experiment used seedling stage, elongation stage, and harvest stage as sampling times for analysis. The sampling times were May 5–7, June 14–16, and July 14–16, respectively. The fresh fully stretched leaves (without large veins) were used for analysis at the different maize stages. Each treatment was replicated three times.

Determination of chlorophyll content, electrolyte leakage, proline, and MDA content

Total chlorophyll content was determined by spectrophotometry. The topmost fully expanded leaves (0.3 g) were sampled and ground into a slurry with 80 % (v/v) acetone/water to extract the pigments (Zhang 2003). The extract was centrifuged at 4000 rpm for 10 min. The supernatant was then diluted with 80 % acetone/water to the appropriate concentration for spectrophotometric analysis at wavelengths of 663 and 645 nm for chlorophyll a and chlorophyll b, respectively.

Electrolyte outflow was checked using the method of Li (2009) and a conductivity meter DDS-11A, China. The uppermost completely extended leaves (0.2 g) were cut into small parts of about 1 cm^2 using a hole punch and added to test tubes containing 20 mL deionized water. To reduce floating, the leaves were covered by some glass fragment. The tubes were maintained at 25 °C for 4 h before electrical conductivity of initial medium (EC1) was determined. Then the samples were placed in a water bath at 100 °C for 15 min to expel all electrolytes. Samples were cooled to room temperature and a final electrical conductivity (EC2) reading was made. EC (%) was computed with the following formula:

$$\mathrm{EC}\ (\%) = \frac{\mathrm{EC1}}{\mathrm{EC2}} \times 100\%$$

Proline was extracted by the sulfosalicylic acid method (Bates et al. 1973). Fresh leaves (0.3 g) were homogenized in 5 mL of 3 % sulfosalicylic acid and then centrifuged at 3000 rpm for 10 min. The test tube samples were then moved to a boiling water bath for 30 min with 2 mL supernatant, 2 mL distilled water, 2 mL glacial acetic acid, and 4 mL acidic ninhydrin. Samples were then quickly cooled using an ice bath to room temperature, and 4 mL methylbenzene was added to extract proline. The absorbance of the extract was measured at 520 nm.

Malondialdehyde (MDA) content was assayed with thiobarbituric acid colorimetry (Shalata and Neumann 2001). A 0.5-g leaf sample was homogenized in 5 mL 10 % TCA. The homogenate was centrifuged at 4000 rpm for 10 min, and the supernatant collected; 2 mL of the supernatant and 2 mL 0.6 % TBA were added and mixed in a test tube. The mixture was heated at 100 °C for 15 min and then quickly cooled in an ice bath to room temperature. After centrifugation at 4000 rpm for 15 min, the absorbance of the supernatant was read at 532 and 450 nm.

Antioxidant enzyme assay

Antioxidant enzyme system components, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in leaves, were determined using a spectrophotometer.

Fully stretched leaves of maize in different growth stages were sampled for enzyme analysis. Each leaf treatment of 0.5 g (avoiding large leaf vein) was placed into a mortar and ground with a pestle in the ice bath. The samples were standardized in 0.05 M PBS (pH 7.8), filtered through four layers of muslin cloth, and centrifuged at 12, 000 rpm for 10 min at 4 °C. The enzyme extracts were used for quantification of SOD, POD, CAT activities following methods described by Li (2009). SOD activity was assayed with nitroblue tetrazolium (NBT); one unit of SOD activity was defined as the amount of enzyme required to cause 50 % inhibition of the reduction of NBT monitored at 560 nm. POD activity was measured using guaiacol, the addition of the enzyme extract started the reaction, and the increase in absorbance was recorded at 470 nm for 5 min. CAT activity was determined by measuring the reduction in the absorbance at 240 nm as a result of H_2O_2 disappearance.

Measurement of plant height and leaf area

Plant height and leaf area data were collected using a meter scale. Plant height is the distance between the soil level and the uppermost leaf. Leaf area was computed with a leaf area meter.

Heavy metal concentration in maize foliage

A 1-g plant sample was placed into a microwave tube with 4 mL concentrated HNO_3 and 2 mL H_2O_2 for 30 min. The sample digestion with H_2O_2-HNO_3 was accelerated with a microwave dissolver (CEM-MARS5, USA). After digestion, samples were transferred to a flask, mixed, and put on hot plate (160 °C) to remove the remaining acid. Samples were then diluted with 5 % HNO_3 to 25 mL and then filtered, and metal determination was performed by inductively coupled plasma-mass spectrometry (Agilent 7700, USA).

Statistical analysis

Comparisons of treatment data were made using analysis of variance (ANOVA) with SPSS 17.0 statistical software and Microsoft Office Excel 2013. The results are expressed as the means±standard deviations (n=3). Tukey's multiple comparison test was used to determine the significance of the differences between the treatments in the same growth stage ($P \leq 0.05$).

Results and discussion

Chemical composition and physical properties of environmental materials

Humic acid (HA) is a type of organic matter (OM) with several active functional groups, such as hydroxyl, carboxyl, and amino (González et al. 2014; Hladký et al. 2013). OM is one of the most influential factors in the control of metal mobility, bioavailability, and toxicity (Williams et al. 2011). Humic acid has been widely used to stimulate soil fertility and decrease metal mobility. In the current study, HA probably acted as a natural barrier to limit the accumulation of Pb and Cd in plants.

SAP is three-dimensional, cross-linked hydrophilic network of polymer chains. The chemical composition of SAP is sodium polyacrylate (PAANa). SAP is widely used in agriculture for its water holding capacity and evaluation of soil physicochemical properties (Devine and Higginbotham 2015). In our study, we added SAP to each combination treatment because of its great water holding capacity. It may also be possible to demonstrate the ability of SAP for immobilization of Pb and Cd in soils due to the chelating ability of functional groups (e.g., $-COO^-$) in the PAANa matrix.

Zeolites are naturally occurring hydrated aluminosilicate minerals. The structures of zeolites consist of three-dimensional frameworks of SiO_4 and AlO_4 tetrahedra. In this study, the major components of zeolite were found to be clinoptilolite, which is the most abundant natural zeolite and has the chemical formula $Na_{0.1}K_{8.57}Ba_{0.04}$ $(Al_{9.31}Si_{26.83}O_{72})\cdot$

19.56H_2O (Galli et al. 1983). The large specific surface area facilitated adsorption of the metal ions and reduced the mobility of heavy metals (Jiang et al. 2012).

Fly ash composite (FC) is a low-cost sorbent for soil remediation (Shaheen et al. 2014). The major components of FC are alumina, silica, ferric oxide, and calcium oxide. FC is alkaline and can neutralize wastewater and adsorption metal hydroxides. However, the use of FC in agriculture is limited because of its low N and P contents, low soil microbial activity, and high pH (Wong and Wong, 1989). Some reports mention and suggest the use of FC as a soil ameliorant for improving physical properties of soil (Shen et al. 2008).

Treatment effects on physiological characteristics of maize

Significant physiological processes of plants, including chlorophyll, proline, and cell membrane permeability, are sensitive to the changes in the external environment (Drost et al. 2007). Physiological variation can therefore reflect the effects of environmental materials on immobilization of heavy metals in contaminated soils.

Effects of test materials on maize chlorophyll content are shown in Fig. 1. A significant decrease was seen in the additive treatment and control CK1 over the entire growth period. Maize chlorophyll was inhibited and damaged after prolonged stress from Pb and Cd. In contrast to single test material, combined test materials had high chlorophyll content. The effect of SAP was seen in treatments (B1, B2, and B3), which enhanced chlorophyll content compared to single test material treatments. This result may be due to the sufficient water supplied for maize growth (Li et al. 2013). In the first two growth stages, treatment C1 had the highest chlorophyll contents of 2.025 and 1.386 mg/g, respectively. At harvest, the highest chlorophyll level was treatment D (0.91 mg/g), which was 2.3 times that of treatment FC. These findings indicated that synergistic effects of the soil additives on the protection of chlorophyll synthesis system occurred under heavy metal stress.

Electrolyte leakage (EL) is a key biochemical index of plants under adverse conditions. Figure 2 shows that EL in maize leaves rose with time in the material treatments. Comparing treatment FC to other single test material treatments, the electrolyte leakage mushroomed from 14.56 to 25.31 %. After prolonged stress in the FC treatment, the cell membranes suffered severe injuries. The average EL in the control CK1 (26.3 %) was 2.86 times greater than treatment CK2 (9.21 %), clearly indicating the cell membrane damage in maize subjected to severe stress without protective environmental materials. A combination of soil additive materials was most effective for reducing injuries to the cell membrane.

Proline is an organic osmolyte that accumulates in plants responding to stress. It can scavenge the reactive oxygen species (ROS) and stabilize protein structure (Verbruggen and Hermans 2008). The influence of soil treatments on leaf proline is shown in Fig. 3. Proline in the different treatments increased from seedlings to plant maturity. Accumulation of proline was closely related to plant resistance (Siripornadulsil et al. 2002). Pb and Cd stress should be responsible for increased proline in plants (Qi et al. 2015). The proline content exhibited non-significant variations among the material treatments during seedling stage but showed significant differences at harvest. Treatments C1 and D had especially low proline levels of 61.87 and 54.57 μg/g, respectively.

Malondialdehyde (MDA) level is an essential parameter used to determine membrane damage (Gallego et al. 1996). The MDA concentration was analyzed to determine the ROS level in response to heavy metal stress. Figure 4 shows the correlation between MDA concentration in leaves and treatments. Compared with control CK1, the application of soil treatments significantly reduced MDA in different maize growth periods. MDA content had a variation similar to proline content in different treatments from seedling to harvest. At harvest, the MDA content in treatment SAP was 2.612 μmol/L, while the MDA content in treatment D was 1.696 μmol/L.

The differences in proline accumulation, together with the decreased MDA concentration, indicate that plants treated

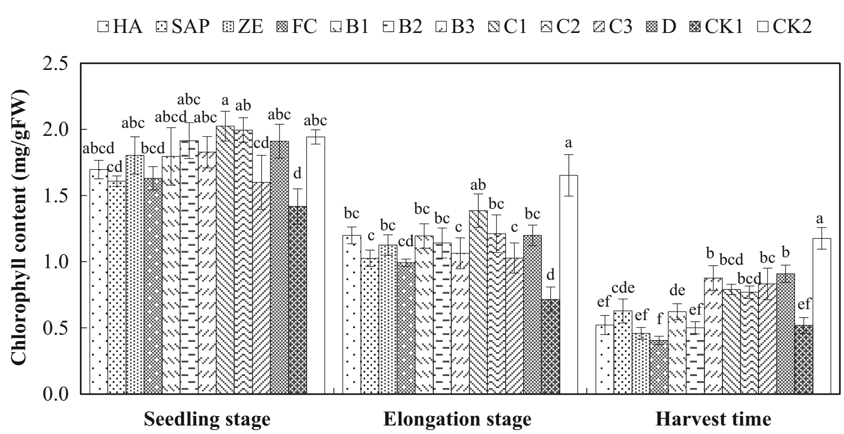


Fig. 1 Influences of different treatments on chlorophyll content in maize leaves. *Error bars* represent the standard deviation of the mean (n=3). Means followed by similar small letters in the same stage are not significantly different at P<0.05 using Tukey's multiple comparison test

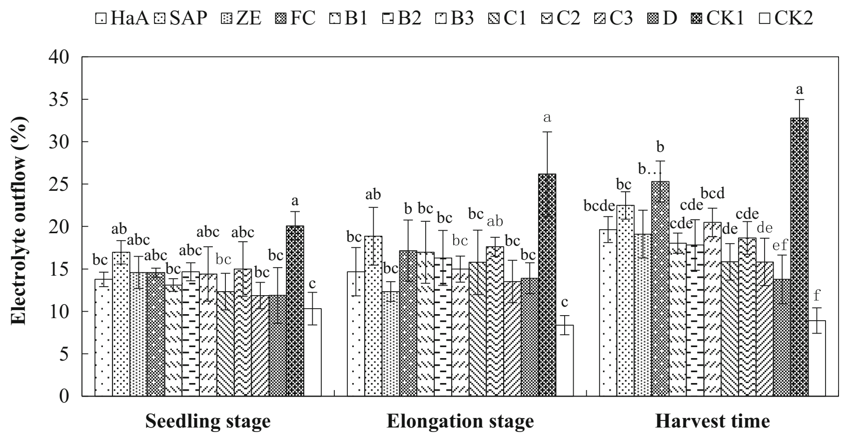


Fig. 2 Influences of different treatments on electrolyte outflow in maize leaves. *Error bars* represent the standard deviation of the mean (n=3). Means followed by similar small letters in the same stage are not significantly different at $P<0.05$ using Tukey's multiple comparison test

with a combination of materials had lower oxidative stress symptoms than a plant treated with only one material under Pb and Cd stress. The result was especially evident in the HA+ZE mixture. SAP and FC alone did not significantly reduce maize injuries. This may be due to the chemical groups of these materials and the variations of soil pH and soil structure (Nayak et al. 2015; Hegazy et al. 2014; Urík et al. 2014; Christl et al. 2001). The differences between single treatment and combination treatment became significant with time. The potential advantages of combinations on immobilization of heavy metals are evident after a long period of stress.

Influences of different treatments on activities of antioxidant enzymes in maize

Toxic levels of heavy metals can interact with several crucial cellular biomolecules, leading to the production of excessive ROS. In response, plants have a repertoire of mechanisms to counteract heavy metal stress (Gallego et al. 1996). Antioxidant enzymes mainly include SOD, POD, and CAT, which regulate the cellular superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), thus inhibiting the production of ^-OH radicals. SOD and CAT play a key role in removal of oxidative stress (Iannelli et al. 2002).

The activities of SOD, POD, and CAT in maize leaves exposed to Pb and Cd stress are demonstrated in Fig. 5a–c. The activities of SOD and POD in material treatments increased at first and then decreased with time. The CAT activity in each material treatment did not differ during maize growth. However, the antioxidant enzyme activities in the control CK1 without any environmental materials decreased with time. The result shows that remarkable differences can be observed between the test material treatments and controls on the antioxidant enzyme activities in maize leaves. In response to heavy metal stress, plant cells have developed antioxidant defense mechanisms to decrease oxidative injury (Kanwal et al. 2014).

However, the enzyme system may be damaged under prolonged stress caused by high levels of heavy metals. In our experiment, the significant decreased activity of SOD and POD in the control CK1 may have resulted from damage to antioxidant enzymes under prolonged stress without protection of environmental materials. Compared with controls, the enzyme activities in heavy metal treatments were low at the seedling stage which may have been due to the immobilization of Pb and Cd by soil additives. While the enzyme activities in CK1 decreased because of enzyme system damage, the material treatment enzymes remained at high levels from elongation stage to harvest, especially in treatments C1

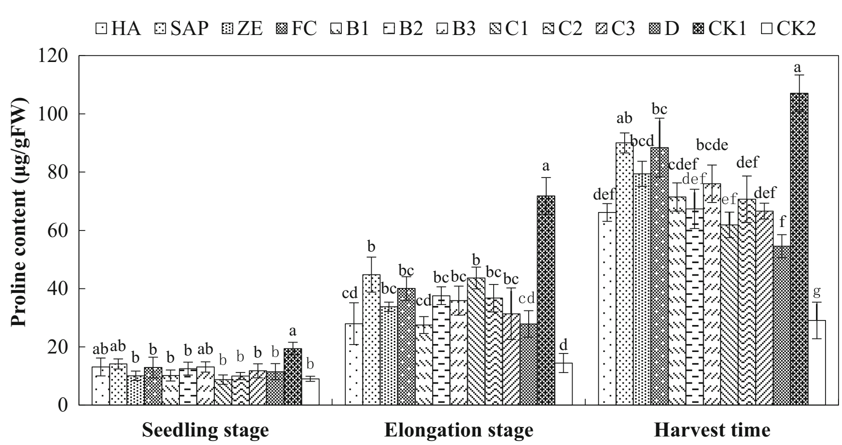


Fig. 3 Influences of different treatments on proline content in maize leaves. *Error bars* represent the standard deviation of the mean (n=3). Means followed by similar small letters in the same stage are not significantly different at $P<0.05$ using Tukey's multiple comparison test

Fig. 4 Influences of different treatments on MDA content in maize leaves. *Error bars* represent the standard deviation of the mean (n=3). Means followed by similar small letters in the same stage are not significantly different at P<0.05 using Tukey's multiple comparison test

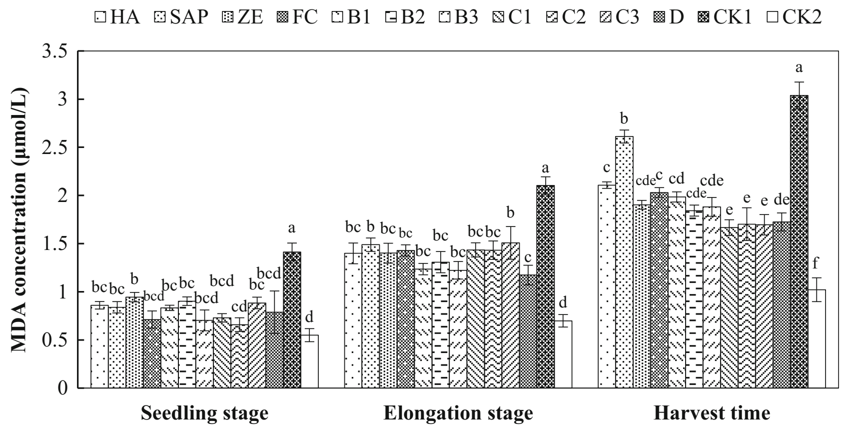
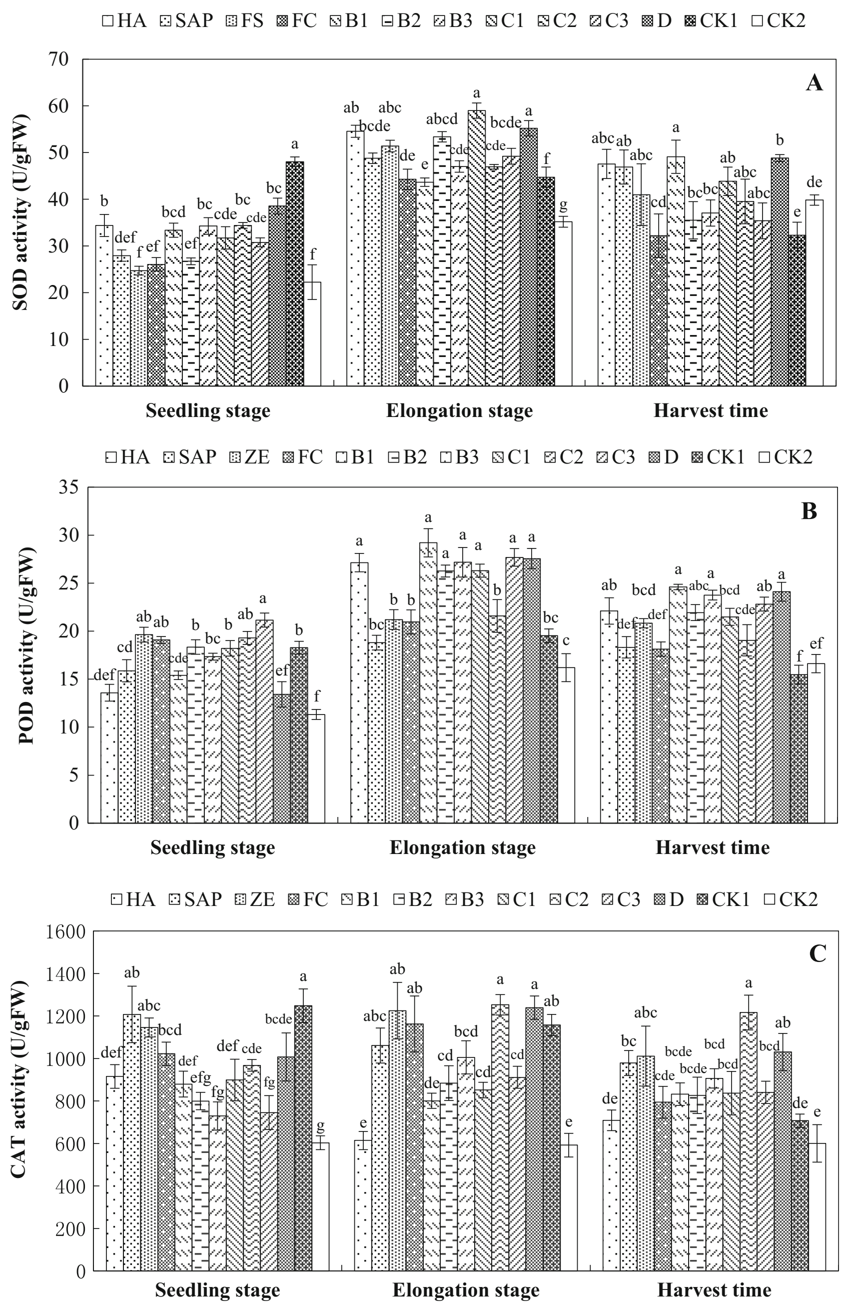


Fig. 5 Influences of different treatments on antioxidant enzymes in maize leaves including SOD (**a**), POD (**b**), and CAT (**c**). *Error bars* represent the standard deviation of the mean (n=3). Means followed by similar small letters in the same stage are not significantly different at P<0.05 using Tukey's multiple comparison test



and D. This suggests that environmental materials can prolong the action time of antioxidant enzymes to reduce the oxidative damage. Combinations of environmental materials can provide a synergetic effect to deactivate heavy metals and reduce their bioavailability to plants.

Morphological variations of maize

Plant height and leaf area are reduced when exposed to the toxic effects of Pb and Cd. Effects of environmental materials on the height and leaf area of plants in contaminated soil are summarized in Fig. 6. In the seedling stage, plant height and leaf area of CK1 were 47.5 cm and 93.12 cm^2, respectively. In CK2, these parameters were 57.6 cm and 121 cm^2, respectively. Pb and Cd toxicity inhibits the growth of many plant species (Gopal and Rizvi 2008) which is consistent with the results of this study. In the elongation stage, treatments C1 and SAP had a relatively high level of plant height. SAP have numerous functional groups for water absorption, which may foster plant height and leaf area development (Li et al. 2013). In this stage, treatment FC had a minimum height of 83 cm. The leaf areas of C1 and D were 308.6 and 310.4 cm^2, respectively. At harvest time, treatment D had the greatest plant height (111.7 cm) and leaf area (461.4 cm^2). Although the maize's morphological indicators in CK2 were higher than those in CK1 from seedling stage to harvest time, no significant differences were observed in plant height among additive treatments. However, leaf area showed obvious differences between treatment CK1 and the other material groups after a long time stress. Treatment D had significant advantages in promoting accumulation of plant biomass.

Heavy metal content in maize foliage

The content of Pb and Cd in maize foliage at harvest is shown in Table 4. Harvest time is the most important stage of plant growth. The ultimate effectiveness of environmental materials on the immobilization of heavy metals is shown at harvest. Pb and Cd levels in CK1 were 42.75 and 14.96 mg/kg, respectively. There was a significant difference between CK1 and the other treatments. A significant decrease of Pb and Cd content in the combination treatment was observed, which may be related to a synergetic effect of the additives. Compared to other single test treatments, treatment FC and HA had low levels of Pb and Cd content, respectively. Treatment D had the lowest content of Pb and Cd in maize foliage. The results showed that combinations of environmental materials, especially treatment D, can significantly inhibit the migration of Pb and Cd to maize foliage.

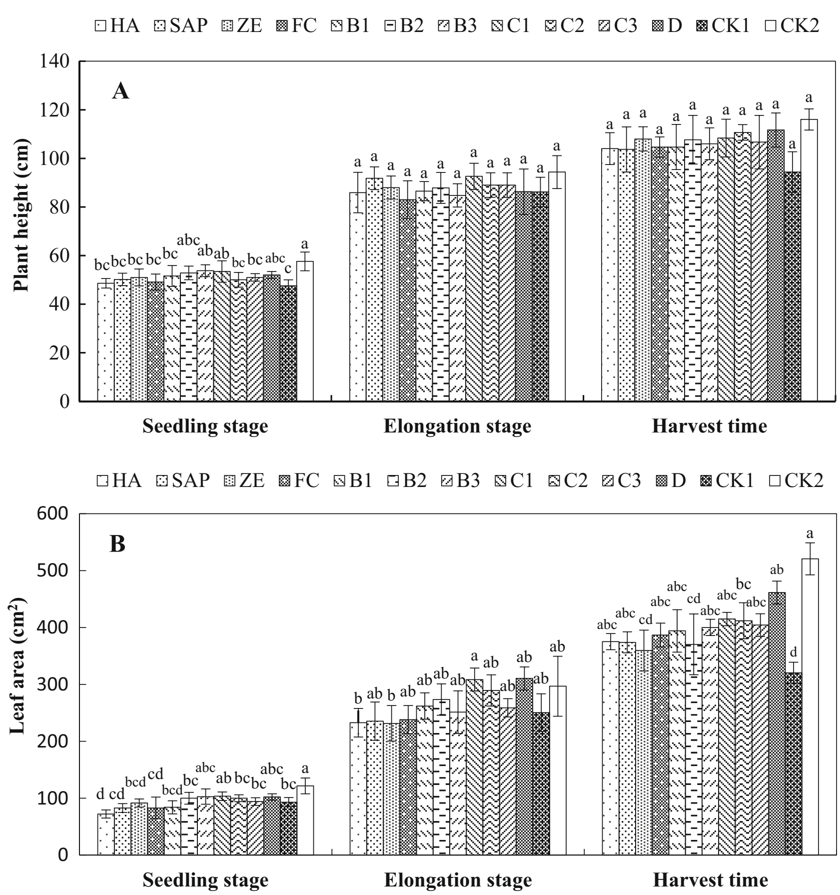


Fig. 6 Influences of different treatments on plant height (**a**) and leaf area (**b**). *Error bars* represent the standard deviation of the mean (n=3). Means followed by similar small letters in the same stage are not significantly different at P<0.05 using Tukey's multiple comparison test

Table 4 Lead and cadmium concentration in maize foliage in harvest time

Treatment	Heavy metal concentration	
	Lead (mg/kg)	Cadmium (mg/kg)
HA	31.75±1.93 cd	10.14±0.59 cd
SAP	37.05±2.00b	13.21±0.70ab
ZE	34.79±2.37bc	11.4±0.75bcd
FC	29.80±1.23d	12.29±0.52bc
B1	33.05±1.55bcd	11.64±1.55bcd
B2	30.28±0.79 cd	11.02±1.04bcd
B3	29.89±1.76 cd	12.22±0.76bc
C1	28.44±0.47d	10.74±1.08 cd
C2	30.07±1.79 cd	10.73±0.51 cd
C3	31.24±1.29 cd	11.19±0.45bcd
D	28.14±1.224d	9.87±0.75d
CK1	42.75±2.73a	14.96±0.43a
CK2	10.41±0.88e	1.06±0.18e

Values are expressed as mean±SD (n=3). Means followed by similar small letters in a column are not significantly different at P<0.05 using Tukey's multiple comparison test

Analysis of mechanisms associated with the test materials

This study focuses on the use of eco-friendly and low-cost materials as potential sorbents for the immobilization of heavy metals. The test materials and their combination showed different levels of ability to immobilize heavy metals, which is closely related to their adsorption mechanisms.

Humic acid (HA), one of the agents selected for this study, has a strong ability to adsorb heavy metals. HA can be divided into two parts including particulate HA (pHA) and dissolved HA (dHA). The pHA can act as a sorbent and dHA can form complexes with metal cations. HA's ability to immobilize heavy metals may be due to the dual effect of pHA and dHA. The chemical composition of SAP is sodium polyacrylate (PAANa). The adsorption capacity of Pb(II) and Cd(II) ions in soil may be attributed to the chelating ability of functional groups (e.g., $–COO^{-}$) in the PAANa matrix, which is consistent with the results of previous reports (Zheng et al. 2010, Yu et al. 2015). The $–COO^{-}$ and –COOH groups indicated a strong propensity to chelate metal ions, which were involved in ion exchange and complexation interactions (Wang et al. 2013). The aluminum ion on natural zeolite (NZ) is small enough to occupy the center of the tetrahedron of four oxygen atoms, and the isomorphous replacement of Si^{4+} by Al^{3+} produced a negative charge in the lattice. The net negative charge was balanced by the exchangeable cation (sodium, potassium, or calcium). The special structure gave NZ a large specific surface area and ion exchange ability for adsorbing the metal ions. Fly ash composite (FC) is an alkaline material that exhibits pH within the range of alkalinity when added to water, and its surface is negatively charged at high pH. Hence, it can be expected that metal ions can be immobilized by precipitation or electrostatic adsorption.

From the results, we can see that the effect of single material on immobilizing Pb(II) and Cd(II) ions become weak over time, which may be attributed to the desorption of metal ions from the materials. The combinations of the test materials may reduce the desorption behavior and prolong the immobilization time. However, in this study, the adsorption abilities of Pb and Cd ions on the test materials and their combinations were also found to be different, which was closely related to factors such as soil pH, contact time, heavy metal concentration, and material dosage. In this way, the effects of different treatments on immobilization of heavy metals may differ under different conditions. This issue should be addressed in an intensive study.

Conclusions

Environmental materials decreased the long-term stress effects of Pb and Cd to maize by reducing biochemical and morphological damage. Under Pb and Cd stress, individual treatments (humic acid (HA), super absorbent polymer (SAP), zeolite (ZE), and fly ash composites (FC)) were less effective than composite treatments with two or more components for promoting plant growth, chlorophyll synthesis, and leaf area and maintaining antioxidant enzyme activities for longer durations. The effectiveness was also reflected by a reduction in the accumulation of proline and MDA in maize leaves and a reduction in the Pb and Cd content in maize foliage. SAP had a positive effect on photosynthesis characteristics and maize growth and showed capability for immobilizing heavy metals, but the abilities decreased with stress duration.

Among the combination soil treatments, C1 (HA+SAP+ZE) and D (HA+SAP+ZE+FC) were especially effective at protecting the biochemical and morphological properties of maize. These combinations could be applied to remediate soil contaminated by high concentration of Pb and Cd. The synergistic effects of different materials were found in different maize growth stages.

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