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Can phosphate compounds be used to reduce the plant uptake of Pb and resist the Pb stress in Pb-contaminated soils?

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Abstract

The effects of different phosphate-amendments on lead (Pb) uptake, the activities of superoxide dismutase (SOD) and the level of malondialdehyde (MDA) in cauliflower (*Brassica oleracea* L.) in contaminated soils with 2500, or 5000 mg P_2O_5/kg soil of hydroxyapatite (HA), phosphate rock (PR), single-superphosphate (SSP) and the mix of HA/SSP (HASSP) were evaluated in pot experiments. Results showed that the Pb concentrations in shoots and roots decreased by 18.3%–51.6% and 16.8%–57.3% among the treatments respectively compared to the control samples. The efficiency order of these phosphate-amendments in reducing Pb uptake was as follows: HASSP≈ HA > SSP ≈ PR. With the addition of SSP, HA and the mix of HA/SSP, the SOD activity in shoot was reduced markedly ($P < 0.05$) compared with that in the control group. For example, the SOD activities in shoot by the treatments of HASSP, SSP, and HA in 5000 mg P_2O_5/kg were found to be only 51.3%, 56.2%, and 56.7%, respectively. Similar effects were also observed on the level of MDA in the shoots with a decrease in 24.5%–56.3%. The results verified the inference that phosphate compounds could be used to reduce the plant uptake of Pb and resist the Pb stress in the plant vegetated in Pb-contaminated soils.

Key words: phosphate amendments; lead; superoxide dismutase (SOD); malondialdehyde (MDA); contaminated soil

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Introduction

Lead (Pb) is one of the most abundant heavy metal pollutants in both aquatic and terrestrial environments. Pb pollution of agricultural soils is one of the most important ecological problems on world scale, it can often accumulate in considerable amounts in the plant tissue and exceed the levels that are toxic to human health before it produces visible phytotoxic effects (Chen, 2002; Bulut and Tez, 2007; Park *et al*., 2007). The lead contamination in the plant is known to affect germination of seeds and exerts a wide range of adverse effects on growth and metabolism of plants (Singh *et al*., 1997; Zhou *et al*., 1999; Pang *et al*., 2002). Our previous tests showed that addition of phosphate (P)-based amendments has proven to be extremely effective as a chemical immobilization treatment for Pb-contaminated soils in China (Zhu *et al*., 2004; Chen *et al*., 2006, 2007). Recently, this P-based approach to the remediation of Pb-contaminated soils has been widely used because of its cost-effectiveness and less disruptive nature (Basta and McGowan, 2004; Cao *et al*., 2004; Chen *et al*., 2007). Significant effort has been made to evaluate the effectiveness of P on *in situ* remediation of contaminated soils and waters (Ma and Rao, 1997; Laperche and Traina, 1998; Hettiarachchi and Pierzynski, 2002; Chen *et al*., 2007). Phosphate minerals, i.e., hydroxyapatite (HA), phosphate rock (PR), single-superphosphate (SSP) can reduce the Pb uptake effectively by the plant and immobilization in various contaminated soils and waters (Laperche and Traina, 1998; Hettiarachchi and Pierzynski, 2002; Zhu *et al*., 2004). It is inferred that these materials can be used to resist Pb stress in contaminated soils by reducing the Pb uptake. Unfortunately, few studies have been conducted on the effects of different P-amendments on the lipid peroxidation and superoxide dismutase of plants induced by Pb stress in contaminated soils, because Pb is considered to be the pre-oxidants inducing oxidative stress, resulting in lipid peroxidation, DNA damage and depletion of sulfhydryls (Weckx and Clijsters, 1997; Pang *et al*., 2002). Antioxidant enzymes such as superoxide dismutase (SOD) play important roles in scavenging reactive oxygen species produced under oxidative stress (Harris, 1992), and thereby protects potential cell injury against tissue dysfunction (Miquel, 1989).

The present study was to evaluate (1) the effects of different phosphate-amendments on the Pb uptake and distribution pattern of Pb by plant; and (2) the effects of P-amendments on the Pb-induced possible induction of

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the oxidative stress and likely alterations in behaviour of the enzymes of antioxidant defense system in vegetable plant (*Brassica oleracea* L.) in Pb-contaminated soil based on the measure of dry weight of shoot and root, uptake of Pb in plant tissues, activities of SOD and the level of malondialdehyde (MDA) content in shoot.

1 Materials and methods

1.1 Soil sample and phosphate treatments

The tested soil was collected from a residential area (0–20 cm in depth) in Fuyang City, Anhui Province, China. The soil was contaminated because of a local Pb smelting factory. The soil sample was air-dried and then ground to pass through a 2-mm sieve for the pot trial. The total Pb and some physicochemical properties in soil were determined using standard methods recommended by the Chinese Society of Soil Science (Lu, 1999). The soil properties included pH 7.76, organic matter 1.67%, Olsen-P 26.7 mg/kg, cation exchange capacity (CEC) 16.7 cmol/kg, and total Pb 297.0 mg/kg.

Phosphate-amendments used in this study were: hydroxylapatite ($Ca_5(PO_4)_3OH$; HA) (particle diameter < 50 μm) derived from bone char, phosphate rock (PR, particle diameter < 133 μm) from Kaiyang Fertilizer Co., Ltd., China, and single superphosphate (SSP). These materials did not contain detectable Pb. Soil amendments were applied to soil at three P levels: 0 (control), 2500, and 5000 mg/kg soil as HA, PR, SSP, and HA/SSP mixture (1:1, *W/W*). The amounts of phosphorus applied are higher than normal agricultural practice, but was as recommended for contaminated soils (Hettiarachchi and Pierzynski, 2002; Zhu *et al*., 2004). The required P amount for each treatment was calculated on the basis of the total P content of the three amendments. All together, there were nine treatments: control (CK), HA/SSP2, PR2, SSP2, HA2, HA/SSP5, SSP5, PR5, and HA5. Each treatment had four replicates. Soil amendments were thoroughly mixed with soil prior to potting. The soil was also supplied with nitrogen (0.2 g N/kg soil as NH_4NO_3) and potassium (0.125 g K/kg soil as KCl) as fertilizers. Polystyrene (12 cm in diameter and 9 cm in height) pots containing 500 g of soil were used in this experiment. Deionized water was added to bring the soil water content to about 60% of maximum water holding capacity (MWHC).

Cauliflower (*B. oleracea* L.) was chosen for this plant test because there is general health concern with heavy metals (Pb) accumulation in leafy vegetables in China. After a 2-week incubation period, ten pre-germinated seeds were sown in each pot. After 7 d emergence seedlings were reduced to four per pot, one for the measurement of SOD and MDA, others for the biomass and Pb content determination. During the experiment, pots were watered with deionized water according to water loss by weighing. The plants were grown in a greenhouse with temperatures range 20–30°C in summer in Beijing, China. Plants were harvested 35 days after emergence.

1.2 Determination of lead content

After harvest, the plants were carefully removed from each pot. Roots were washed thoroughly to get rid of adhering soil particles, followed by quick wash with deionized water. Plants were then divided into root and shoot parts. One fresh plant (about the mean size) was taken for the measurement of SOD and MDA. Other plants were oven-dried at 60°C for 72 h, the weight of shoots and roots was recorded. For the analysis of Pb in plant tissues, plant powder (1.0 g dried weight) were digested in 15 mL $HCl/HNO_3/HClO_4$ (3:1:2, *V/V/V*) mixture at 140°C until the solution became clear. The concentration of Pb in the digest solution was determined by inductively coupled plasma optical emission spectrometer (ICP-OES) (Optima 2000, Perkin Elmer. Co., USA). Quality control samples included standard reference materials of soil (GSS-6, China National Center for Standard Materials) and plant (GSV-4).

1.3 Superoxide dismutase

Fresh shoots and roots of 0.5 g was homogenized in 50 mmol/L phosphate buffer (pH 7.0) containing 1% polyvinyl pyrrolidine. The homogenate was centrifuged in a refrigerated centrifuge at 18000 $\times g$ for 15 min. The supernatant was used for enzyme assays. All operations in the preparation of enzyme extract were carried out at 4°C. The activity of SOD was assayed according to photochemical method described by Giannopolitis and Ries (1977). One unit of SOD activity was defined as the amount of enzyme resulting in 50% inhibition of the rate of *p*-nitro blue tetratzolium chloride reduction at 560 nm. The reaction mixtures (3.0 mL) are listed in Table 1. Riboflavin was added as the lastly, the test tubes were shaken and placed 30 cm below a light source (30 W fluorescent lamps). The reaction took 30 min and was stopped after 30 min by switching-off the lights. The tubes were then covered with black cloth. The blank was reaction mixture without exposure to light. The absorbance was measured at 560 nm. Log absorbance 560 nm was plotted as a function of the volume of enzyme extract used in the reaction mixture. The volume of the enzyme extract corresponding to 50% inhibition of the reaction was considered as one enzyme unit and expressed as U/g fresh weight per min (U/g fw).

Table 1 Superoxide dismutase (SOD) assay kit: schedule for reaction mixture (NBT-assay)

Initial solution	Volume (mL)	Conc. in mixture
Phosphate buffer 0.05 mol/L (pH 7.8)	1.5	
Methionine 130 mmol/L	0.3	13 mmol/L
Nitro blue tetrazolium 750 μmol/L	0.3	75 μmol/L
EDTA-Na_2 100 μmol/L	0.3	10 μmol/L
Riboflavin 20 μmol/L	0.3	2.0 μmol/L
Enzyme extract	0.1*	
Deionized water	0.2	
Total volume	3.0	

* Phosphate buffer solution 0.1 mL is taken as control.

1.4 Measurement of lipid peroxides

The level of lipid peroxidation in shoot and root was expressed as the content of MDA measuring by the method of Health and Packer (1981) with some modification. The enzyme extraction was treated with 10% TCA (trichloracetic acid) containing 0.7% thiobarbituric acid (TBA). The mixture was incubated in 95°C water for 30 min, then cooled in an ice bath and centrifuged at 18000 ×*g* for 15 min. The absorbance of the supernatant was read at 532 nm and corrected for unspecific turbidity by subtracting the absorbance at 600 nm. The blank was 0.7% TBA in 20% TCA. The concentration of lipid peroxides in terms of MDA level using an extinction coefficient of 155 mmol/(L·cm) and expressed as μmol/g fresh weight (μmol/g fw).

1.5 Statistical analysis

Mean difference comparisons among different treatments including the analysis of variance were conducted using SPSS version 12.0 software (SPSS Inc., USA) and differences were reported at $P < 0.05$. Data was expressed as mean ± SD.

2 Results

2.1 Effects on plant biomass and uptake of Pb

The addition of phosphate amendments increased the shoot biomass significantly except for PR2 treatment, while root biomass increased slightly in some treatments as compared to the control treatment (Table 2). The biomass of the shoots and roots increased with the P addition level, the reasons may involve Pb toxicity decreasing or phosphorus nutrition increment after phosphate amendments addition. It is possible that the above two mechanisms could work together. SSP and HA treatments had obvious effect on shoot and root biomass increment, while PR treatments had marginal effect on root biomass increment as compared to the control.

The application of phosphate amendments significantly reduced the concentrations of Pb in the plant root growing in the contaminated soil, and the concentrations of Pb in the plant shoot decreased significantly with all amendments except for PR2 and HASSP2 treatments (Table 3). Pb concentrations in both shoot and root decreased with increasing rates of phosphate application with an exception of SSP in root. In the control treatment, mean Pb concentration in plant root was 60.2 mg/kg, and it was reduced substantially to 25.7 mg/kg in the HA5 treatment. In the treatment of HASSP5, the Pb concentration in shoot was only 51.9% of that in control. The reduction of Pb concentrations in the plant shoots of HASSP5, PR5, SSP5, and HA5 treatments were 52.0%, 32.9%, 38.1%, and 48.0%, respectively. Generally, the efficiency of the phosphate amendments in reducing Pb content in shoot followed the order: HASSP≈ HA > SSP ≈ PR.

The absorbed lead was localized to a greater extent in root than in shoot (Table 3), however, the obtained concentration of Pb in root and shoot did not immediately yield quantitative information on the translocation of Pb from the root to shoot after the remediation. Such information can, be obtained by defining a root to shoot concentration ratio (R/S ratio), as the concentration of Pb in plant root divided by that in plant shoot. HASSP5 had the largest R/S ratio (3.21) among the treatments, which implied a considerably more difficult shoot translocation of Pb in HASSP5 treatment than other amendments (Table 3). The smallest R/S ratio was observed for SSP2 treatment (1.82), which indicated that Pb in root was obviously more available for translocation to shoot (edible part). But no significant differences ($P < 0.05$) were observed between the treatments and control.

2.2 Effect of P-amendments on the activities of SOD in shoots

Changes in the activities of SOD and the MDA content in the shoot and root after P-amendment treatments showed similar trends, here only the result for shoot are shown in Figs. 1 and 2. The effects of different phosphate amendments on the activities of SOD in shoot of cauliflower (*B. oleracea* L.) under lead stress were shown in Fig. 1. The activity of SOD of the shoot in the control was 22.4 U/g fw. Addition of SSP, HA, and the mix of HA/SSP reduced the activity of SOD of the shoot significantly ($P < 0.05$), the activities of SOD in the shoot of HASSP5, SSP5, and HA5 were only 51.3%, 56.2%, and 56.7% as that in control treatment respectively, but no significant difference ($P < 0.05$) was found among the P-amendments at the same level. The activity of SOD of shoot decreased

Table 2 Biomass of cauliflower (*Brassica oleracea* L.) in Pb-contaminated soil in pot culture receiving various phosphate amendments (mean±SD, g/pot dw)

Treatment	Shoot	Root
CK	1.09 ± 0.05 e	0.18 ± 0.02 c
HASSP2	1.45 ± 0.13 cd	0.20 ± 0.02 bc
HASSP5	1.79 ± 0.10 ab	0.31 ± 0.03 a
PR2	1.13 ± 0.07 e	0.19 ± 0.03 c
PR5	1.54 ± 0.06 cd	0.23 ± 0.03 bc
SSP2	1.61 ± 0.12 bc	0.25 ± 0.05 b
SSP5	1.85 ± 0.19 a	0.32 ± 0.05 a
HA2	1.40 ± 0.10 d	0.25 ± 0.03 b
HA5	1.78 ± 0.11 ab	0.31 ± 0.03 a

Data with the same letters in the same column are not significantly different ($P < 0.05$).

Table 3 Lead concentration in plant tissues by the treatment of different P amendments (mg/kg)*

Treatment	Shoot	Root	R/S ratio
CK	25.2 ± 2.5 a	60.2 ± 5.0 a	2.42 ab
HASSP2	20.6 ± 4.2 abc	45.0 ± 4.2 bc	2.24 ab
HASSP5	12.2 ± 3.7 e	36.2 ± 3.4 d	3.21 a
PR2	21.5 ± 3.7 ab	50.1 ± 5.5 b	2.42 ab
PR5	16.9 ± 5.2 bcde	41.4 ± 4.5 cd	2.67 ab
SSP2	19.0 ± 3.0 bc	33.6 ± 1.7 d	1.82 ab
SSP5	15.6 ± 2.0 cde	35.3 ± 7.6 d	2.27 ab
HA2	18.5 ± 3.0 bcd	38.4 ± 7.5 cd	2.11 ab
HA5	13.1 ± 3.5 de	25.7 ± 5.7 e	2.17 b

Data with the same letters in the same column are not significantly different ($P < 0.05$).

with the increase of phosphate addition level. Although PR treatments reduced the activity of SOD in shoot, no significant ($P < 0.05$) difference was found as compared with that in the control (Fig. 1).

2.3 Effect of P-amendments on MDA content in shoot

The effects of phosphate-amendments on the MDA content in shoot of cauliflower (*B. oleracea* L.) under lead stress in contaminated soil are shown in Fig. 2. After the phosphate-amendments for 49 d, the contents of MDA in shoot was reduced by 24.5%–56.3% as compared with that of control (7.01 μmol/g fw), and the negative relation could be seen among the contents of MDA in shoot and the phosphorus addition level. The most significant effect was observed in HASSP5 treatment (3.06 μmol/g fw). All P-amendments showed marked effects ($P < 0.05$) on the reduction of MDA contents in shoot except for PR2 treatment (Fig. 2), which was similar to that on the activity of SOD of the plant shoot. In summary, the efficiency of the P-amendments on the reduction of MDA contents and the SOD activity in shoot was followed the order: HASSP ≈ HA ≈ SSP > PR.

2.4 Relationship between Pb content and the activities of SOD and MDA content

Correlation coefficients suggested that there were significant positive correlations ($r = 0.560^{**}$, $P < 0.01$) between Pb concentrations and the activities of SOD in the shoot (Fig. 3a). Similarly, Pb concentrations presented significant positive correlations ($r = 0.606^{**}$, $P < 0.01$) with the contents of MDA in shoot (Fig. 3b). These relationships were in agreement with the fact that under the lead stress, plants are readily to uptake more lead, however, heavy metals such as Pb are known to cause oxidative

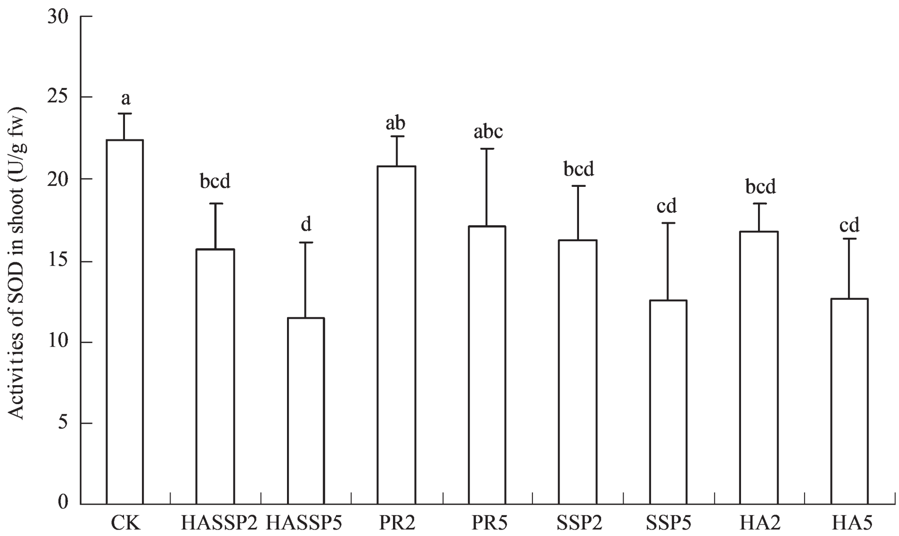

Fig. 1 Effect of P-amendments on the activity of SOD in plant shoots (mean ± SD, $n = 4$). The bars with the same letter indicate no significantly difference ($P < 0.05$).

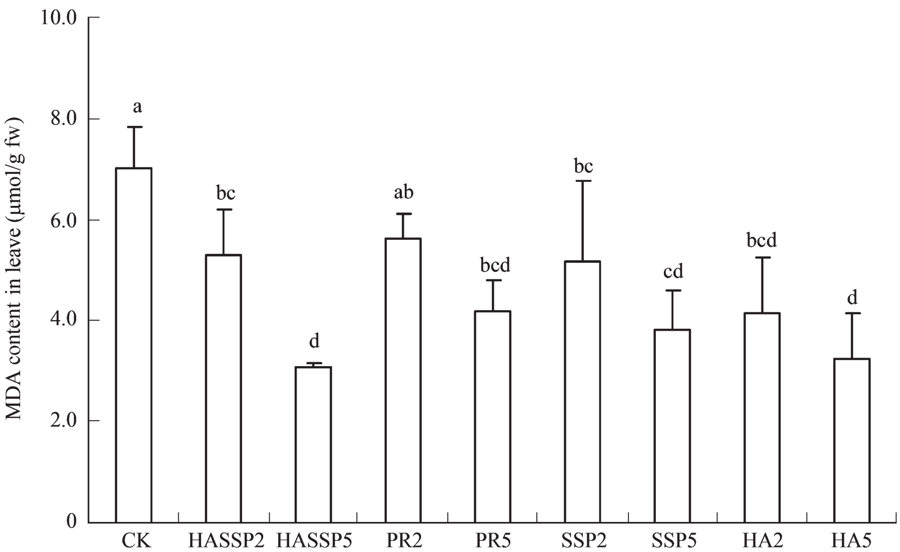

Fig. 2 Effects of P-amendments on the contents of MDA in plant shoots (mean ± SD, $n = 4$). The bars with the same letter indicate no significantly difference ($P < 0.05$).

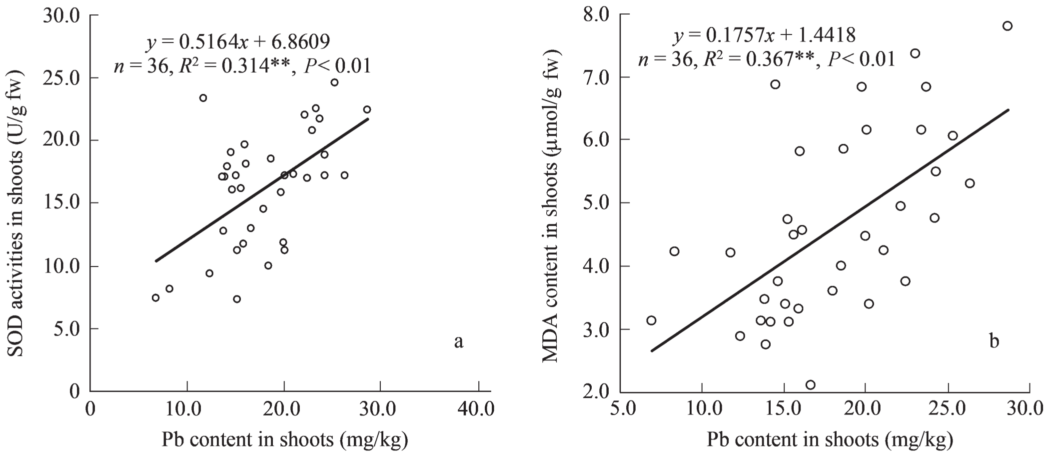

Fig. 3 Relationship between Pb concentration and the activity of SOD (a) and the content of MDA (b) in plant shoots.

damage to plants either directly or indirectly by triggering an increased level of production of reactive oxygen species (ROS) (Lee *et al*., 1976; Pang *et al*., 2002; Alavala *et al*., 2005), resulting in the increased activities of SOD and MDA content in plant tissues in contaminated soils.

3 Discussion

Heavy metals Cd, Pb, Al, Zn, and Cu are known to affect germination of seeds, plant growth, metabolism and induce oxidative stress in certain plant species (Cakmak and Horst, 1991; Chongpraditnum *et al*., 1992; Mazhoudi *et al*., 1997; Prasad *et al*., 1999; Malecka *et al*., 2001; Shah *et al*., 2001). The most common effect of Pb toxicity in plants is stunted growth and alteration in the activity of many key enzymes of various metabolic pathways (Pang *et al*., 2002; Alavala *et al*., 2005), however, the antioxidant enzymes are important cellular defense system against oxidative stress, for example, SOD plays major role in the intracellular defense against oxygen radical damage to aerobic cells under stress condition (Lee *et al*., 1976; Harris, 1992). Pb stress in the present study resulted in a high activity of SOD and high content of MDA in the plant (Figs. 1 and 2), the high superoxide dismutase activity and thereafter the high content of MDA could possibly be the result of both a direct effect of Pb stress in plant tissues and an indirect effect mediated via an increase in levels of $O_2^{\bullet}$ (Chongpraditnum *et al*., 1992). This study showed that lead concentration of shoot and root of the plant was reduced markedly after the addition of different phosphate-amendments when compared to controls (Table 1). The positive correlations between the Pb content and the activities of SOD/the MDA content in plant tissues could probably verify the inference that the phosphate-amendments decreased the plant stress induced by lead toxicity, in other words, the phosphate-amendments could enhance the plant resist to lead stress in contaminated soils. However, the magnitude of decrease in the lead/MDA contents and the activities of SOD of the plants were dependent on the sources of phosphorus even with the same phosphorus addition level. This phenomenon indicated that phosphate specific effect is important in reducing Pb bioavailability in contaminated soils. In this study, HA, SSP and the mix of HA/SSP had the better effects than PR treatment in reducing the plant uptake of Pb with the same addition level and our previous study also found that the addition of HA exhibited the best effect in reducing of Pb accumulation in plant tissues among the phosphate-amendments (Zhu *et al*., 2004). The reasons for the difference of the remediation effects and translocation of Pb in the plant (R/S concentration ratio) may involve several mechanisms of immobilization of Pb by phosphates in soils, such as adsorption/desorption, precipitation, rhizosphere modification and physiological reactions etc. One of the key processes of Pb immobilization by phosphates-amendments is the formation of pyromorphite or other compounds with low solubility, resulting in the transformation of Pb from nonresidual fractions to residual fraction (Hettiarachchi and Pierzynski, 2002; Basta and McGowan, 2004; Cao *et al*., 2004; Chen *et al*., 2006). Results from our previous study confirmed that the addition of HA substantially increased the residual fraction of Pb in soils (Zhu *et al*., 2004; Chen *et al*., 2006), which corresponded to the reduction of Pb concentrations in plants tissues, while PR had weaker effect for reducing Pb bioavailability and plant uptake of Pb than HA, this probably due to the differences of solubility or the ability for adsorption of Pb between HA and PR in the soil, or both of them. Because this contaminant is directly involved in the human food chain, information on the concentration level, transfer of Pb and the enzyme reactions in plants will provide important data for the environmental risk assessment at such sites after *in situ* remediation using phosphate amendments. To investigate the cause of the above differences, further study need to be conducted.

4 Conclusions

The results confirmed that the input of phosphate to soil could help the plant to face the oxidative stress provoked by the presence of Pb in contaminated soils. In the presence of phosphate-amendments, the amounts of the uptake of Pb by the plant, SOD and MDA in shoot and root were significantly reduced compared to the control. In general, the effect of different phosphates on plant uptake Pb and the consequent improvement on plant tolerance of oxidative stress followed the order: HA/SSP≈HA > SSP≈ PR. These results verified the inference that Pb bioavailability could be reduced and the resistance to Pb stress could be enchanced in Pb-contaminated soils by phosphate compounds.

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