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Influences of major nutrient elements on Pb accumulation of two crops from a Pb-contaminated soil

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ABSTRACT

To know about the effect of major nutrient elements on various forms of Pb and metal extraction, a greenhouse experiment was conducted to assess the effects of various major nutrient elements on Pb accumulation in two crops (*Spinacia oleracea*, SO and *Sonchus arvensis*, SA) in Changchun, China. Results indicated that, for SO, the Pb concentrations in both shoots and roots had no difference with increasing nutrients except for low nutrient treatment (1/2H). For SA, high nutrient treatments (2H and 3H) resulted in higher Pb concentrations in roots than low and standard nutrient treatments (1/2H and C), but high Pb concentration in shoot appeared in low and highest nutrient treatments (1/2H and 3H). The nitrogenous nutrient treatment (2N) had the most effect of increasing Pb concentrations in roots of SO and SA. The potassic and phosphorus nutrient treatments (2K and 2P) had little effect on the Pb concentrations in plant tissues for SO. Pb concentration in SO was lower than SA. Because of the higher total biomass in SO than SA, the ability to Pb accumulation in SO was better than SA. Sequential extraction results indicated that the addition of soil amendments transform soil Pb from bioavailable fractions to non-bioavailable fraction substantially. The results suggest that nitrogen fertilizer for SO and phosphorus fertilizer for SA are the most effective materials for the remediation of Pb-contaminated soils, and increase the tolerance of crops to Pb contamination.

1. Introduction

Pb is one of the most widespread metals found on the earth's surface [1]. Human activities such as mining, smelting, burning of fossil fuels, dumping of municipal sewage sludge, and the manufacture of pesticides and fertilizers are the primary sources [2]. Pb is a biologically nonessential element, highly toxic to humans as well as animal reproduction and development, and known to adversely affect plant seed germination, nutrient assimilation, photosynthesis and growth [3–9]. The US Center for Disease Control and Prevention has recognized Pb as the most common and serious environmental hazard to children [10]. In the European Union, restrictions on the maximum concentrations of Pb allowed in several agricultural crops were recently enacted into law [11]. Although some sources of Pb contamination have been reduced worldwide, environmental emission of Pb is still increasing in many countries [12]. This issue is especially important in China since coal, the traditional fuel for cooking and heating, emits various metals including Pb during the burning process [13,14].

Today, more and more people are calling for immediate action on the magnitude of Pb pollution problem in soils. Many factors affect Pb transport and accumulation in plant–soil systems, such as soil pH, soil redox potential, cation exchange capacity and fertilizer application, etc. Chemical mobilization is a promising technique to increase the mobility of contaminants in the soil. It involves the addition of chemical and mineralogical materials to the contaminated soils to increase the solubility and bioavailability of metals through plant absorption and/or precipitation. Previous studies have evaluated several types of soil amendments to mobilize Pb [15–18] such as EDTA, exogenous humic substances, fertilizers and soil colloids [19–23]. However, in recent years the use of persistent aminopolycarboxylic acids, such as EDTA, has caused scientists and policy makers alike to oppose the entire technology of enhanced phytoextraction. This compound is resistant to biodegradation and is, therefore, characterized by high environmental persistence. Its prolonged presence in the soil, combined with its ability to chelate and mobilize heavy metal, dramatically increases risks of leaching.

The nutrients (N, P and K) are indispensable to plants. The interaction between accumulation of heavy metals and nutrients in plants is rather complicated, and shows synergistic and antago-

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Table 1
General soil properties and total metal content of the dredged sediment-derived soil used in the pot experiments; pH: actual soil acidity, OM: organic matter content; TN: total nitrogen; TP: total phosphorus; intervals denote standard deviation (mean ± S.E., $n = 4$).

pH	7.20 ± 0.10	TN (mg kg^{-1})	19 ± 0.33
Clay (%)	5	TP (mg kg^{-1})	90 ± 0.75
Silt (%)	9	K (mg kg^{-1})	358 ± 1.70
Sand (%)	86	Pb (mg kg^{-1})	5.61 ± 0.16
OM (%)	1.93 ± 0.18	Fe (mg kg^{-1})	650 ± 9
CEC (cmol kg^{-1})	3.98 ± 0.23	Mn (mg kg^{-1})	719 ± 5

nistic effect [24]. Inorganic fertilizer is the preferred method for nutrient delivery because its cost is relatively low, and the addition of nitrogenous, phosphorus and potassic fertilizers has become a normal agricultural practice. Mobilization of Pb is highly increased by inorganic fertilizers containing $NH4^+$ even at low pH [11,19]. Phosphates have been found to decrease the positive charge, and increase the negative charge or cation exchange capacity of soil [25,26], which would enhance heavy metal sorption by soil, which immobilize Pb in Pb-contaminated soil. Due to the different distributions of Pb among chemical fractions, each with different bioavailability, there is usually a poor relationship between plant uptake and total content of Pb in soil [27–29]. A water soluble and exchangeable form of Pb is generally considered to be bioavailable for plant uptake, and pH influences the transformation between exchangeable form and other forms. Fertilizers can alter soil properties such as pH and surface charge, or directly react with heavy metal ions in soil. Accordingly, the ability of ammonium sulfate to extract the metals with inducing a strong acidification of the medium is a very desirable characteristic, and hydroxyapatite and phosphate rock amendments can increase the stability of soil Pb, so it decreases the uptake of plants [11,12]. All these effects can result in changes in the forms of heavy metals, but very little attention has been paid to the effect of N, P, and K combined fertilizer on the mobilization of Pb.

The objectives of this study were to investigate the effect of various nutrient addition amendments on Pb accumulation by crops (*Spinacia oleracea*, abbreviate SO; and *Sonchus arvensis*, abbreviate SA) growing in contaminated soils, and its relation to changes in Pb speciation under various nutrient addition amendments. Specific attention was directed at accumulation of Pb in plants and the distribution of different Pb forms in the soil.

2. Materials and methods

2.1. Soil sample and amendments

The soil substrate used in our experiments was a dredged sediment-derived surface soil from a local riverbed in Changchun, China. Physical and chemical characteristics of the soil sample are reported in Table 1. Samples were air dried, ground to pass through a 2-mm sieve and stored in plastic containers until use. The Pb nitrate solution, $Pb(NO_3)_2$, was added to the air-dried sample at the concentration of 500 mg Pb kg^{-1} (7500 mg Pb pot^{-1}), which was the value of environmental quality of class 3 soil of national standards (the critical value of lead in soil to ensure production of agriculture and normal growth of plants) [30]. To enable the added heavy metal salts to reach a steady state the treated soils were wetted for 2 weeks by adding deionized water to maintain 60% of the soil water-holding capacity. Soils were then dried at room temperature for 2 weeks. The artificially contaminated soil was subjected to three cycles of wet and dry processes before pot experiments were conducted [31].

2.2. Crops

Two types of crops, *Sp. oleracea* (SO) and *Se. arvensis* (SA), with high annual biomass yield, strong root systems, short growing periods, and low planting costs were chosen for greenhouse pot experiments. Both crops are widespread in riverbeds of northern China and there is a general health concerning Pb accumulation in these crops. Pb is distributed in the soil of riverbeds adjacent to some industries in China. Therefore both crops could potentially be used for soil remediation.

2.3. Nutrient treatments

The amount of nutrition in each treatment was calculated on the basis of the standard Hoagland solution. This was a completely randomized design with four replications and seven nutrient treatments: C (CK, full standard Hoagland solution. N, P, and K were 17, 1, and 6 mmol l^{-1}, respectively), 1/2H (half strength Hoagland solution. N, P, and K were 8.5, 0.5, and 3 mmol l^{-1}, respectively), 2H (double strength Hoagland solution. N, P, and K were 34, 2, and 12 mmol l^{-1}, respectively), 3H (triple strength Hoagland solution. N, P, and K were 51, 3, and 18 mmol l^{-1}, respectively), 2N (double strength nitrogenous component on the basis of the standard Hoagland solution. N, P, and K were 35, 1, and 6 mmol l^{-1}, respectively), 2P (double strength phosphorus component on the basis of the standard Hoagland solution. N, P, and K were 17, 2, and 6 mmol l^{-1}, respectively), 2K (double strength potassic component on the basis of the standard Hoagland solution. N, P, and K were 17, 1, and 12 mmol l^{-1}, respectively). Full standard Hoagland solution contains (in mmol l^{-1}) 5 KNO_3, 5 $Ca(NO_3)_2 \cdot H_2O$, 1 NH_4NO_3, 2 $MgSO_4$, 1 KH_2PO_4, mixture of 0.02 $FeSO_4 \cdot 7H_2O$ and 0.02 NaCl, 0.01 $MnCl_2 \cdot H_2O$, 0.045 H_2BO_3, and (in μmol l^{-1}) 0.8 $ZnSO_4$, 0.3 $CuSO_4 \cdot 5H_2O$, and 0.1 $NaMoO_4 \cdot 2H_2O$. The addition of Pb nitrate solution, $Pb(NO_3)_2$, induced to the increase of the concentration of 68 mg N kg^{-1} (1020 mg N pot^{-1}) in the soil. In contrast to the nutrient solution (714 mg N kg^{-1} in full standard Hoagland solution), the amount of N (added with Pb) was low, so which did not accommodate in the calculation of the amount in each nutrient treatment.

Polystyrene (22 cm in diameter and 15 cm in height) pots containing 15 kg of treated soil were used in this experiment. Nutrient solutions were added once a week to pots, maintaining the soil moisture content at 20% (w/w) by weighing [12]. Ten pre-germinated seeds of each crop were sown in each pot at 2 cm depth. Each pot was thinned to five seedlings per pot 8 days after seedling emergence. During the experiment, pots were watered every 3 days with deionized water, according to observed water loss determined by weighing. The plants were grown in a greenhouse with temperatures between 20 and 25 °C in summer. Plants were harvested 2 months after seedling emergence.

The shoot:root ratios of Pb-pools were calculated as follows:

$$\text{shoot : root ratio of Pb-pools} = \frac{\text{Pb accumulation of shoot}}{\text{Pb accumulation of root}}$$

2.4. Chemical analysis

All soil samples were analyzed with four replications for general soil properties and total metal content (Table 1). To determine actual soil pH, 10 g of air-dried soil was allowed to equilibrate in 50 ml of deionized water for 24 h. Soil pH was determined in 1:5 soil/water suspensions after 0.5 h with a combination pH electrode (Model DDB-30A, Beijing, China), calibrated using pH 6.88 and pH 9.18 standards. Organic matter was determined using the Walkley-Black method described by Allison [32]. The grain size distribution of the soil samples was determined using laser diffrac-

Table 2
European Community standard, measurements and testing procedure used in this study.

Step	Fraction	Method
A	Water soluble fraction, exchangeable fraction and carbonate bound fraction	0.11 mol l^{-1} HOAc, 40 ml, 20 °C, shaking overnight
B	Fractions bound to hydrous oxides of Fe and Mn	0.1 mol l^{-1} $NH_2OH{\cdot}HCl$ (pH = 2 with HNO_3) 40 ml, 20 °C, shaking overnight
C	Organically bound fraction	8.8 mol l^{-1} H_2O_2 (pH = 2–3 with HNO_3), 10 ml, room temperature for 1 h Additional 8.8 mol l^{-1} H_2O_2 (pH = 2–3 with HNO_3), 10 ml, 85 °C for 1 h Additional 1 mol l^{-1} NH_2OAc (pH = 2 with HNO_3), 50 ml, 20 °C, shaking overnight

tometry (Coulter LS200, Miami, USA) [33]. Total nitrogen (TN) was determined using a Kjeltec 2300 Analyzer Unit (Foss Tecator AB, Sweden). Total phosphorus (TP) was determined using the molybdenum blue method [34]. Concentrations of K, Pb, Fe and Mn in the soil were analyzed using flame atomic absorption spectrometry (SpectrAA-220FS, Varian, USA).

Plants were carefully removed from each pot at harvest, and roots were washed thoroughly to remove adhering soil particles by a quick wash in deionized water. Soil in each pot was stored for Pb concentration and pH analysis. Plants were then divided into roots and shoots and oven-dried at 60 °C until their weight was constant. For the analysis of Pb in plant tissues, 1 g of dried tissue was dissolved in 15 ml $HCl/HNO_3/HClO_4$ (3:1:2, v/v/v) at 150 °C until the solution became transparent [12]. The resultant solutions were filtered and diluted to 25 ml in volumetric flasks and stored at 4 °C prior to analysis using a flame atomic absorption spectrometer (SpectrAA-220FS).

2.5. Sequential extraction of Pb in soil with different treatments

Sequential extraction was performed for air-dried soil samples from each pot (1 g in 40 ml polyethylene centrifuge tubes) according to the procedures of the European Community standards [35]. Step A included a water soluble fraction, an exchangeable fraction and a carbonate bound fraction; while step B consisted of fractions bound to hydrous oxides of Fe and Mn, and step C included an organically bound fraction (Table 2). After each extraction, separation was done by centrifugation at 10,000 rpm for 15 min. Pb concentrations in each fraction were determined by flame atomic absorption spectrometry.

2.6. Statistical analysis

The Pb concentration in shoots and roots, and soil pH were analyzed with a one-way ANOVA (SPSS 10.0) within each species for the effect of nutrients. Pb element pools for the whole plant and individual tissue types (shoots and roots) were determined based on their concentration and biomass within each pot. The effect of nutrients on whole plant biomass, individual tissue biomass, and their Pb element pools were then statistically analyzed. Mean separation was done using LSD at $p \leq 0.05$.

3. Results

3.1. General properties of the soil

The general properties of the soil are presented in Table 1. The soil was near neutral (pH 7.2), the CEC values of the soil were low (3.98 cmol(c) kg^{-1}), and the soil samples were mostly composed of sand. The soil samples contained low amount of organic matter. The total N, P, and K contents of soil were extremely low. The background Pb content was low, so the effect on experiment was ignored nearly.

3.2. Soil pH

Soil pH was significantly affected by nutrient treatments and the trend was similar between SO and SA ($p < 0.0001$) (Fig. 1). Compared to the C treatment, for both SO and SA, the 2N treatment significantly reduced soil pH by around 0.8 and 1.4 units, respectively; the 1/2H, 2H, 3H and 2P treatments had little effects on soil pH. In comparison to the C treatment, the 2K treatment remarkably increased soil pH by about 0.6 units for SO ($p = 0.0028$).

3.3. Pb speciation as affected by nutrient treatments

Pb speciation in soils sown with SO and SA showed similar trends (Figs. 2 and 3). Application of nutrient increasing treatments resulted in a decline in the A fraction of Pb, with the application of 2H and 3H having the lowest level of A fraction of Pb. The levels of A fraction were similar for treatments with 2N and 2P for both crops. Application of 2K and 1/2H had little influence on the B fraction of Pb, while the application of 2H and 3H enhanced the B fraction of Pb considerably ($p = 0.0043$, $p = 0.0200$). Concentrations of the C fraction of Pb were also increased by the addition of nutrient treatments for both species, such as 2H, 3H, 2N and 2P (Fig. 3). The application of 1/2H had the lowest concentration of the C fraction of Pb for SA plants, but for SO plants there was no difference between 1/2H and C treatments. Among the six treatments, the soils supplied with 2H, 3H and 2P had the highest concentration of the C fraction of Pb.

3.4. Plant biomass

Patterns of biomass allocated to individual tissue types remained different within both species across the nutrient treat-

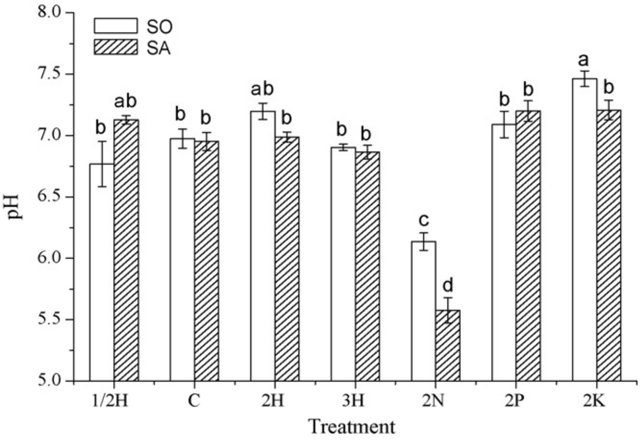

Fig. 1. Soil pH as affected by nutrient treatments. (SO) *Sp. oleracea*; (SA) *Se. arvensis*; (1/2H) half strength Hoagland solution; (C) full standard Hoagland solution; (2H) two strength Hoagland solution; (3H) three strength Hoagland solution; (2N) two strength nitrogenous component on the basis of the standard Hoagland solution; (2P) two strength phosphorus component on the basis of the standard Hoagland solution; (2K) two strength potassic component on the basis of the standard Hoagland solution. Error bars are S.E.

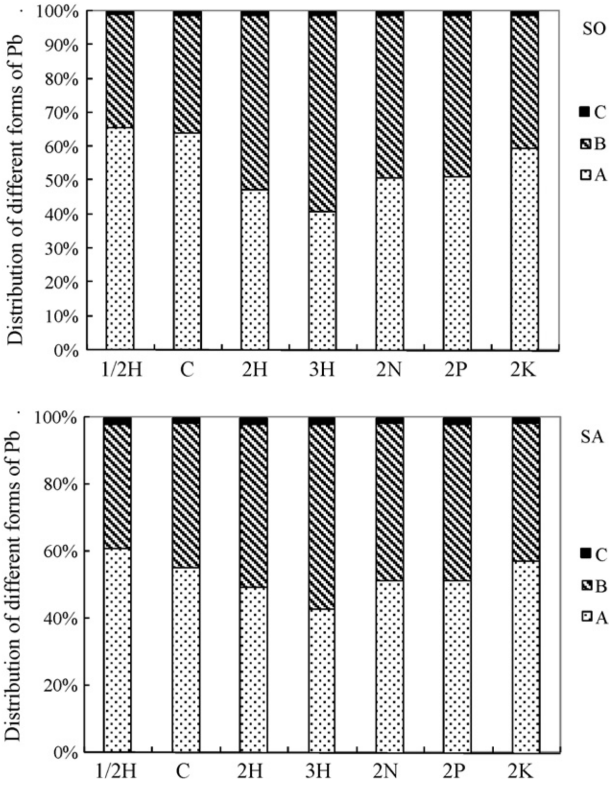

Fig. 2. Speciation of Pb in soil with various treatments. (SO) *Sp. oleracea*; (SA) *Se. arvensis*; (1/2H) half strength Hoagland solution; (C) full standard Hoagland solution; (2H) two strength Hoagland solution; (3H) three strength Hoagland solution; (2N) two strength nitrogenous component on the basis of the standard Hoagland solution; (2P) (two strength phosphorus component on the basis of the standard Hoagland solution; (2K) two strength potassic component on the basis of the standard Hoagland solution. A, B and C stand for various forms of Pb in the soil as indicated in Table 2.

Table 3
Shoot and root biomass per plant of two crops grown in Pb-contaminated soil in pot culture receiving various amendments (g plant^{-1} of dry weight). Means with the same letters are not significantly different at $p < 0.05$.

Treatment	*Spinacia oleracea* (SO)		*Sonchus arvensis* (SA)	
	Shoot	Root	Shoot	Root
C	8.99b	1.91a	0.09d	0.11c
1/2H	9.81b	1.03c	0.22bcd	0.94bc
2H	15.62a	1.76ab	0.38bc	1.15b
3H	17.88a	1.94a	0.48ab	0.92bc
2N	16.75a	1.27bc	0.19cd	0.24bc
2P	17.62a	1.39abc	0.70a	2.50a
2K	10.32b	0.97c	0.10cd	0.28bc
$LSD_{0.05}$	3.98	0.61	0.29	0.94

Table 4
Tissue Pb concentrations of two crops grown in Pb-contaminated soil in pot culture receiving various treatments (mg g^{-1} of dry weight). Means with the same letters are not significantly different at $p < 0.05$.

Treatment	*Spinacia oleracea* (SO)		*Sonchus arvensis* (SA)	
	Shoot	Root	Shoot	Root
C	0.17a	0.39b	0.64b	0.11d
1/2H	0.19a	0.77a	0.74a	0.10d
2H	0.25a	0.28b	0.59b	0.20b
3H	0.17a	0.35b	0.79a	0.21b
2N	0.15a	0.77a	0.73a	0.26a
2P	0.19a	0.44b	0.34d	0.22b
2K	0.16a	0.38b	0.50c	0.15c
$LSD_{0.05}$	0.13	0.27	0.07	0.03

ments. Shoot biomass was greater than root biomass for SO plants, in contrast to SA plants. Total biomass of SO plants was higher than that of SA plants. Compared to the C treatment, the application of 2H, 3H, 2N and 2P treatments had significant impact on the shoot biomass of SO plants ($p = 0.0012$, $p = 0.0003$, $p = 0.0301$, $p = 0.0137$), while 1/2H and 2K treatments had no significant effect on plant biomass, and 1/2H, 2N, and 2K treatments decreased the root biomass comparing with C treatment ($p = 0.0045$, $p = 0.0173$, $p = 0.0033$) (Table 3). For SA plants, application of 2H, 3H and 2P considerably increased the shoot biomass ($p = 0.0232$, $p = 0.0293$, $p = 0.0187$), and the amounts of root tissues were comparable among the nutrient treatments except for 2H and 2P. Compared with the C treatment, 2H and 2P markedly increased the root biomass for SA ($p = 0.0043$, $p = 0.0005$).

3.5. Pb concentration in plants

Pb concentrations were lower in shoots than in roots of SO, and this differed from similar measurements in SA (Table 4). In the case of SO, the addition of nutrients had no influence on Pb concentrations in roots ($p > 0.05$) except for the treatments with 1/2H and 2N ($p = 0.0036$, $p = 0.0106$), and also on Pb concentrations in shoots ($p > 0.05$). Compared with the C treatment, the addition of nutrients enhanced Pb concentrations considerably in roots in SA plants, except for the treatment with 1/2H. Pb concentrations increased only for the treatments with 1/2H, 3H and 2N in shoots ($p = 0.0234$, $p = 0.0202$, $p = 0.0329$), but decreased significantly for treatments with 2P and 2K ($p = 0.0102$, $p = 0.0074$). In contrast to C treatment, for SO, total Pb uptake increased after application of 1/2H treatment and decreased after addition of 2N treatment, and vice verse for SA.

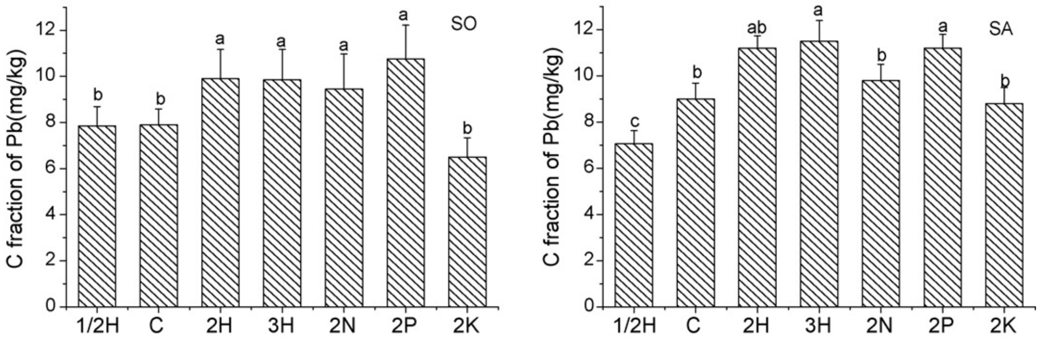

Fig. 3. Changes in C fraction of Pb in soils with various treatments. (SO) *Sp. oleracea*; (SA) *Se. arvensis*; (1/2H) half strength Hoagland solution; (C) full standard Hoagland solution; (2H) two strength Hoagland solution; (3H) three strength Hoagland solution; (2N) two strength nitrogenous component on the basis of the standard Hoagland solution; (2P) two strength phosphorus component on the basis of the standard Hoagland solution; (2K) two strength potassic component on the basis of the standard Hoagland solution. Means with the same letters are not significantly different at $p < 0.05$. Error bars are S.E.

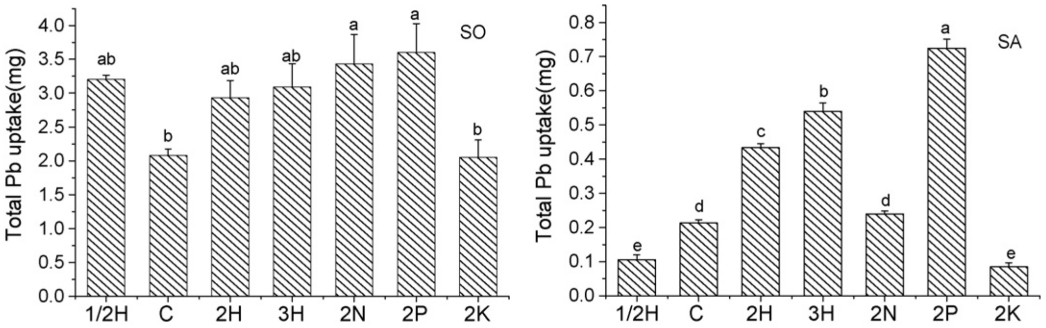

Fig. 4. Total Pb uptake by two crops grown with various soil treatments in pot culture. (SO) *Sp. oleracea*; (SA) *Se. arvensis*; (1/2H) half strength Hoagland solution; (C) full standard Hoagland solution; (2H) two strength Hoagland solution; (3H) three strength Hoagland solution; (2N) two strength nitrogenous component on the basis of the standard Hoagland solution; (2P) two strength phosphorus component on the basis of the standard Hoagland solution; (2K) two strength potassic component on the basis of the standard Hoagland solution. Means with the same letters are not significantly different at $p < 0.05$. Error bars are S.E.

For SA, 2K treatment decreased total Pb uptake, whereas had no difference for SA ($p > 0.05$).

Total plant uptake of Pb (shoot + root) is shown in Fig. 4. For SO, nutrient treatments 2N and 2P had higher Pb uptake than C treatment ($p = 0.0105$, $p = 0.0081$). For SA, nutrient treatments had significant influence on total Pb uptake. The treatments of 2H, 3H and 2P enhanced Pb uptake in tissues significantly ($p = 0.0051$, $p = 0.0016$, $p = 0.0014$), and, conversely, the treatments of 1/2H and 2K greatly reduced uptake of Pb ($p = 0.0082$, $p = 0.0066$). In contrast to C treatment, for SO, total Pb uptake increased after application of 1/2H treatment and decreased after addition of 2N treatment, and vice verse for SA. For SA, 2K treatment decreased total Pb uptake, whereas had no difference for SA. In general, SO plants had higher total Pb uptake than SA. For both species, high Pb uptake was found in the treatments of 2H, 3H and 2P.

3.6. Shoot:root ratios of Pb-pools

Except 2P treatment in SA, all shoot:root ratios of Pb-pools were greater than 1 in both plants, which showed that the Pb-pools in shoot exceeded root (Fig. 5). Further, SO had a much higher shoot:root ratios of Pb-pools than SA in C, 2H, 3H, 2P, and 2K treatments, indicating much greater ability on the accumulation of Pb into photosynthetic tissue. The highest and lowest shoot:root ratios of Pb-pools were in 1/2H and 3H treatments in SO, respectively. But for SA, it is generally constant with above nutrient treatments. For SO, 2N treatment clearly decreased the ratios, but the influence did not occur in 2P and 2K treatments, and for SA, the ratios decreased in 2P treatment only.

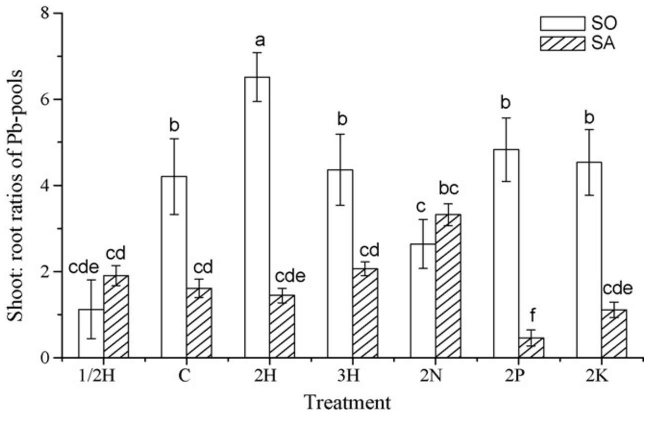

Fig. 5. Shoot:root ratios of Pb-pools by two crops grown with various soil treatments in pot culture. (SO) *Sp. oleracea*; (SA) *Se. arvensis*; (1/2H) half strength Hoagland solution; (C) full standard Hoagland solution; (2H) two strength Hoagland solution; (3H) three strength Hoagland solution; (2N) two strength nitrogenous component on the basis of the standard Hoagland solution; (2P) two strength phosphorus component on the basis of the standard Hoagland solution; (2K) two strength potassic component on the basis of the standard Hoagland solution. Means with the same letters are not significantly different at $p < 0.05$. Error bars are S.E.

4. Discussion

Soil contamination with Pb exists in many agricultural soils, and plant ingestion is a major pathway of amendments. This can be achieved by adding various agents to the soil to increase Pb mobility. Results from the current study confirmed that Pb immobilization was relative to nutrient treatments, because in contrast to C treatment, high nutrient treatments (2H and 3H) appeared to increase the amount of the organically bound fraction of Pb in the soil for SO, but only 3H treatment for SA (Figs. 2 and 3). Compared to the C treatment, Pb immobilization increased in 2N and 2P treatments for SO, but only with 2P for SA, because of increasing the organically bound fractions (Fig. 3). One of the keys to Pb mobilization is the addition of N, P, K nutrient treatments with low solubility, resulting in the transformation of Pb among different fractions [24,36–38]. It was also observed that the application of nutrient treatments induced changes in soil pH [19,27,39]. Generally, urea supply results in a rise in soil pH by 0.02–0.53 pH units above treatments with no urea [19]. In acid soil, urea will be hydrolyzed. If the NH_4^+ produced by urea is not subjected to nitrification or uptake by the plant, soil pH will rise. Nitrification is largely influenced by soil pH. It proceeds slowly below a soil pH of 6.0, and not at all below a pH of 5.0 [40]. And application of NH_4-containing fertilizers could lead to pH decline in soil with pH higher than 6.0 in the presence of plants [41,42]. Soil pH changes could also contribute to the overall effects of Pb mobilization by soil treatments. However, the percents of A fraction (bioavailable Pb) in 2H, 3H, and 2N treatments with higher contents of NH_4^+, were less than C and 1/2H treatments for both plants, it may be explained by Pb mobilization increased strongly as pH decreases below 4.5–3.5 [11]; above these values, mobilization are mainly controlled by P fertilizers. The pH in 2N treatment was merely 5.6, so NH_4-containing fertilizers did not mainly affect Pb mobilization in this study. Much interesting results were found by Schmidt [11], who tested the effect of ammonium sulfate vs. calcium nitrate as N fertilizers on heavy metal accumulation of willow. They found that willow can accumulated 2–3 times heavy metal after ammonium fertilization than nitrate fertilization. The anion NO_3^- in the soil existed denitrification. The soil samples in this study were mostly composed of sand with favorable aeration condition, denitrification was markedly restrained, so the effect of anion NO_3^- on mobilization can be ignored in this study. Furthermore, parts of A fraction of Pb was leached away into the lower soil, which was markedly different from the rhizosphere soil sample in this study. Similar to previous observations [12,19], addition of P can decrease the bioavailability of Pb in soil. In soil rich in iron oxides, P could act as a medium between the soil oxide surfaces and absorbed heavy metals, thus favoring the specific adsorption of heavy metals [19]. So the increase of B fraction of Pb may be due to the increase of Fe–Mn oxides bound form. Furthermore, it should be noted that application of P and N fertilizers not only changes the speciation and bioavailability of heavy metals, but also provides plant nutrients and increases plant biomass substantially. The competitive exchange between K and Pb ions on soil surfaces results in Pb bioavailability increasing [19]. However, under the present experimental conditions, 2K had less effect on increasing Pb bioavailability and plant uptake of Pb; this was probably due to a slight soil pH increase after the application of 2K. In our study, the soil was slightly alkaline (pH = 7.2) (except 2N), which may affect the overall effectiveness of Pb immobilization. High soil pH in this study may also reduce the effectiveness of 2K in respect to increasing Pb bioavailability [12]. It was expected that in acidic soils, 2K may have been more effective in controlling Pb accumulation in plants.

The water soluble fraction, exchangeable fraction and carbonate bound fraction of Pb are generally considered to be bioavailable for plant uptake [12,43], and pH influences the transformation between exchangeable form and other forms. Low organic association of Pb may be due to the extremely low organic matter in soil (Table 1). These results were similar to the observations made by Tu et al., who show that the organically associated Pb was very small (<2.2%) with low organic matter in soil (5.7 g kg^{-1}) [19]. The analysis of the relationship between Pb speciation and Pb concentrations in plant tissues showed that for SO plants, 95% of the variation in shoot Pb concentrations can be explained by the variations in the water soluble fraction, exchangeable fraction and carbonate bound fraction of Pb and soil pH, and the relationship can be described by the following equation:

$$[\mathrm{Pb}]_{\mathrm{shoot}} = 1.887 + (1.395\mathrm{E}\text{-}2)[\mathrm{Pb}]_{\mathrm{A}} - 0.24\mathrm{pH}\,(p < 0.05) \qquad (1)$$

where $[\mathrm{Pb}]_{\mathrm{shoot}}$ is Pb concentration in shoot; and $[\mathrm{Pb}]_{\mathrm{A}}$ is Pb concentrations of water soluble fraction, exchangeable fraction and carbonate bound fraction in soil; pH is the pH in contaminated soil. While for SA plants, Eq. (1) could only explain 70% of the variation in shoot Pb concentrations, implying that the different plant species had different ability to modify the bioavailability of Pb in the soil.

Generally, most inorganic agents used for phytoextraction reduce the soil pH [11]. In this study, the concentration of Pb in both plants were highest in 2N treatment because of the lowest pH, and vice versa at 2K treatment for SO. However, because of the toxicity of soluble aluminum and/or calcium and magnesium deficiency, the growth of most crops is restrained with decreasing pH and is hampered if the soil pH is below 4 [44]. So according to our study, there may be not correlation between pH and Pb accumulation.

Differences in growth were observed among the treatments. Whole plant biomass was greater for SO than SA under identical edaphic nutrient treatments in the greenhouse. An enhancement in biomass in response to the nutrient treatments was found for both species, and this enhancement was distributed relatively equally among tissue types. Phosphorus addition could reduce the toxicity of heavy metal to the growth of SO. Biomass of SO increased with the increase of P concentration in the soil. A reduced Pb toxicity could mean the formation of Pb phosphate complexes, which would thus reduce bioavailability [12]. Despite the lack of statistical difference, SA plants appeared to experience a greater degree of increase in biomass than SO plants (Table 3). We found that SA in 1/2H treatment has higher biomass than C treatment, despite of no statistical difference in both plants. One imaginable explanation may be that SA widely distributed in sterile soil in China, whereas, in the low nutrient condition, it may be able to grow well in disturbed habitats because of an ability to tolerate a given stress, but there are the markedly growth variation of SA.

Pb concentrations of both plants were still elevated at the end of the growing season and differences in Pb allocation were also observed among the treatments. In the soils with nutrient treatments, the shoots of SA had greater concentrations of Pb than shoots of SO at the end of the growing season, nevertheless, in contrast with the pools of Pb owing to distinction of biomass. In SO plants, significantly more of Pb uptake was allocated to aboveground tissues than to belowground tissues. In general, Pattern of Pb distribution within plants was relative to the plant species. Different species of plants possess widely varying abilities to tolerate Pb and tolerance can be a result of several different mechanisms for survival and growth. Plants which are highly tolerant to metal contamination commonly store toxic elements in the vacuoles of cortical tissue of roots outside the endodermis or in cell walls, thereby excluding the metals from uptake to aboveground tissue [7]. The mechanisms of metal tolerance in plants include metal exclusion and accumulation. Of the entire Pb burden experienced by both plants, SO was able to restrict over 20% to its root tissues, whereas 45% of Pb in SA was found in root. Such metal immobilization in root cells, implies an exclusion mechanism [45]. Therefore, it is likely that this mechanism is more efficient for SA, especially in high phosphorus treatment, which appears to be more tolerant to toxic effects. This may account for a more severe inhibition of growth in SO in Pb-contaminated soil. However, the tolerance of each plant to Pb can be better correlated with different nutrient treatments. For SO, the tolerance increased in the low nutrient and high nitrogenous nutrient treatment, but only increased in the high phosphorus nutrient treatment for SA, and decreased in high nitrogenous nutrient treatment. The disparate tolerance of plants resulted in difference of Pb accumulation from both SO and SA. Therefore, Pb accumulation relied on the choice of appropriate crop species and varieties. And various soil amendments are only a supplementary approach to enhancing heavy metal accumulation of plants. Among all treatments, great attention was paid to the effect of 2P treatment on Pb concentration and Pb accumulation in plant tissues. We found that Pb accumulations in plant tissues were high in 2P treatment, though Pb concentrations in plant tissues were not very high. The explanation of this contradiction may be that 2P treatment can boost dry matter biomass production because of nutrition suitability for plants.

This fundamental difference between both species in response to Pb contamination indicates that metal export into food webs or the water body should be greater in stands of SO plants than SA plants. Although the density of biomass in the pots of this greenhouse study was low, the patterns found here were in general agreement with other field studies on Pb concentrations in SA [46]. A further observation related to belowground processes in plants grown on Pb-contaminated soils was supported by seasonal and age patterns observed in the studies of other metals (Hg in Heller and Weber [47]; As, Cr, Cu, Fe, Mg, Pb, in Luque et al. [48] and Windham et al. [7]). These results suggest that in Pb-contaminated soil, the replacement of SA with SO may reduce metal bioavailability by sequestering a lesser proportion of its metal burden in belowground tissues because of transference of contaminants from roots to soil.

Results obtained in this study suggest that in Pb-contaminated soils high levels of N, P, and K fertilizers should be used for vegetable production, whereas, the N fertilizer for SO and the P fertilizer for SA are the most effective materials for the remediation of Pb-contaminated soils, because they increased the tolerance to Pb-contamination and promoted crops' growth.

5. Conclusions

This greenhouse experiment demonstrated that the application of nitrogen and phosphorus amendments other than kalium amendments could effectively increase Pb accumulation by two crops. Major nutrient elements increased Pb accumulation mainly because the biomass and the tolerance to Pb-contamination increased. Variations in shoot Pb concentrations can be partly explained by the variations in soil-exchangeable and carbonate bound form of Pb and soil pH. It is recommended that under excessive contamination with Pb and low organic matter, nitrogen and phosphorus fertilizer should be applied to remediate Pb-contaminated soil with non-hyperaccumulator plants.

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