Journal of Environmental Sciences Vol.15, No.6, pp.836—840, 2003

ISSN 1001—0742
CN11-2629/X

Article ID: 1001-0742(2003)06-0836-05 **CLC number**: X171; S511.06 **Document code**: A

Elemental sulfur effects on Pb and Zn uptake by Indian mustard and winter wheat

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Abstract: A pot experiment was conducted to investigate the influence of elemental sulfur to contaminated soil on plant uptake by a heavy metal hyperaccumulator, Indian mustard(*Brassica juncea*) and a field crop, winter wheat(*Triticum. aestivum*). Elemental sulfur(S) with different rates was carried out, they were 0(S_0), 20(S_{20}), 40(S_{40}), 80(S_{80}), and 160(S_{160}) mmol/kg respectively. Extra pots with the same rates of S but without plants were used for soil sampling to monitor pH and $CaCl_2$-extractable heavy metal changes. The results showed that S enhanced phytoextraction of Pb and Zn from contaminated soil. Application S effectively decreased soil pH down to 1.1 as the most at the rate of S_{160}. The concentrations of $CaCl_2$-extractable Pb and Zn in soil and uptake of Pb and Zn by the plants were increased with soil pH decreased. A good correlation between $CaCl_2$-extractable Pb/Zn and soil pH was found(R^2_{Pb} = 0.847 and R^2_{Zn} = 0.991, n = 25). With S application, soil $CaCl_2$-extractable Pb and Zn concentrations, concentration of Pb and Zn in plants and the amount of removal by plant uptake were significantly higher than those without S. Under the treatment of S_{160}, the highest $CaCl_2$-extractable Pb and Zn were observed, they were 4.23 mg/kg and 0.40 mg/kg, 2.7 and 2.0 times as that of the control(S_0) respectively. At the highest rates of S(S_{160}), both Indian mustard and winter wheat reached the highest uptake of Pb and Zn. The highest Pb concentrations in wheat and Indian mustard were 32.8 mg/kg and 537.0 mg/kg, all 1.8 times as that of the control, and the highest Zn concentrations in wheat and Indian mustard were 215.5 mg/kg and 404.0 mg/kg, 2.4 and 2.0 times as that of the control respectively. The highest removals of Pb and Zn from the contaminated soil were 0.41 mg/pot and 0.31 mg/pot by Indian mustard in the treatment of S_{160} through 50 days growth.

Keywords: elemental sulfur; Pb; Zn; uptake; contaminated soil

Introduction

Lead and zinc are the most common heavy metal contaminants in the environment. Human activities such as mining, smelting, dumping of municipal sewage sludge, manufacturing and using battery are the primary causes. Lead(Pb) is a nonessential element in metabolic processes and may be toxic or lethal to organisms when absorbed in certain amounts. Zinc(Zn) is a necessary element in metabolic processes, but it may be toxic to organisms when it is higher in environment, especially in organisms. Because Pb and Zn could bring potential hazard and widespread contamination, a good approach that use to clean up Pb and Zn at minimal costs with the fewest environmental side effects should be found(Jung, 1997; Martinez, 2000; Markus, 2001; Lim, 2002).

In recent years, phytoremediation, especial phytoextration has been suggested as a novel clear-up technology(Salt, 1995; Brennan, 1999; Bunzl, 2001; Lock, 2001). In phytoextraction, bioavailability of metals is a crucial factor limiting metal uptake by plants from the contaminated soil. In order to increase heavy metals availability to plants, two approaches have been used, one is adding synthetic chelating agents(Blaylock, 1997), such as EDTA, NTA, DTPA, another is decreasing soil pH(Salt, 1995; Chlopecka, 1996; Blaylock, 1997). Decreasing soil pH is adapt to neutral or slightly alkaline soil, because the sorption behaviour of many metals changes significantly pH from 7 to 5.5, and that is the optimum pH range for plant growth in agriculture(Brümmer, 1983).

Soil pH may be decreased by application of acid-producing fertilizers, such as ammonium chloride, or by use of minerals or organic acids, but these methods may result in undesired accumulation of corresponding effects, which may negatively affect soil fertility, soil structure, or may lead to groundwater pollution.

In order to avoid some of these limitations, the use of elemental sulfur to enhance the solubility of heavy metals in soils has been suggested. Certain groups of acidophillic soil bacteria, predominantly the genus *Thiobacillus* in soil, can oxidize S and change soil pH(Lee, 1988; Tichý, 1997; Kayser, 2000). Application of S to increase the solubility of Cd and Zn has been studied(Tichý, 1997; Kayser, 2000). The goal of this study was to investigate whether S amendment could also be used to enhance the solubility of Pb and Zn in contaminated soil and enhance accumulation of Pb and Zn in plants.

Foundation item: The National Natural Science Foundation of China(No. 40171087) and the Chinese Academy of Sciences as a key project in the strategy of the Knowledge Innovation (KZCX2-401-01-1); * Corresponding author

1 Materials and methods

1.1 Soil

Soil material(0—20 cm depth) in this study was collected from a recycle battery factory in Taihe, Anhui Province, China. The soil was air dried and screened passing through a 0.5 cm sieve. The physicochemical characteristics of the soil are presented in Table 1.

1.2 Plant material and cultivation

Indian mustard(*Brassica juncea*) and winter wheat(*Triticum aestivum*. L) were sown at a density of 15 plants per pot and thinned to 8 individuals one week after germination. The experiments were carried out in a greenhouse at a 14 h(24℃)/10 h(16℃) day/night cycle. Water content of the soil was adjusted to approximately 60% of the water holding capacity with deionized water. Plants were grown for 50 d. All the experiments were carried out in triplicates.

Table 1 Physicochemical characteristics of the soil

Soil type	Yellow brown earth
pH(1:2.5 soil/0.01 mol/L $CaCl_2$)	7.11
CEC, cmol/kg	6.05
Clay, %	25.1
Silt, %	42.8
Sand, %	32.1
Total Pb, mg/kg	31000
Total Zn, mg/kg	480
0.01 mol/L $CaCl_2$ extractable Pb, mg/kg	1.59
0.01 mol/L $CaCl_2$ extractable Zn, mg/kg	0.201

1.3 Elemental sulfur treatments

The soil was fertilized with 80 mg/kg P_2O_5(KH_2PO_4), 120 mg/kg K_2O(KCl and KH_2PO_4) and 120 mg/kg N(NH_4NO_3), then divided into 5 parts and thoroughly mixed with 0(S_0), 20(S_{20}), 40(S_{40}), 80(S_{80}), and 160(S_{160}) mmol S per kg soil respectively, and filled into pots(500 g/pot). The soil was allowed equilibration for a period of 14 d in a greenhouse. Extra pots with same rates of S but without plants were used for soil sampling to monitor soil pH and $CaCl_2$-extractable heavy metal changes. All treatments were carried out in triplicates.

1.4 Soil and plant sampling and analysis

In soil experiments, soil samples were collected by cylindrical soil sampler 10 d intervals throughout the experiments. Soil samples were sieved with a 2 mm nylon sieve. Soil pH was measured using a sample with 1:2.5 soil/0.01 mol/L $CaCl_2$ ratio(Koen, 2001); cation exchange capacity(CEC) and particle size were measured with standard methods (Institute of Soil Science, CAS, 1978), 0.01 mol/L $CaCl_2$ extractable Pb and Zn were determined by atomic absorption spectrophotometry (AAS) or inductively coupled plasma emission(ICP-AES), for total soil Pb and Zn the procedure was digesting soil samples with a mixture of concentrated HNO_3 : $HClO_4$: HF(3:1:1), and measuring by AAS. All the soil analysis was carried out in triplicates. The harvested plant materials were rinsed with deionized water, oven dried at 70℃ for 24 h, ground with a mill, digested with a mixture of concentrated HNO_3 : $HClO_4$: HF(3:1:1), and finally measured by AAS.

1.5 Statistical analysis

Analyses of variance(ANOVA) were applied on all data and correlation between soil pH and $CaCl_2$-extractable Pb/Zn contents was calculated. Least significant difference(LSD) multiple comparison procedure was also employed to further elucidate differences between means($\alpha = 0.05$).

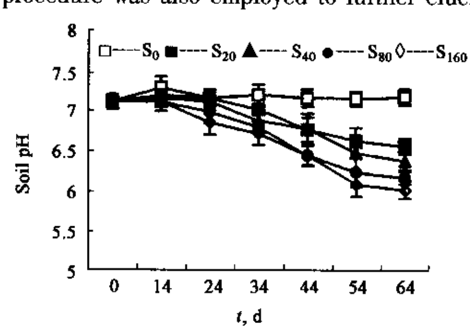

Fig.1 Changes of soil pH(mean ± *SE*) in the different levels of application of S

2 Results

2.1 Soil pH

Soil pH was decreased in all the treatments with the application of S, and the soil pH of control(S_0) had little changes(Fig.1). The soil pH at the treatments of S_{20}, S_{40}, S_{80}, S_{160} was decreased 0.6, 0.7, 1.0, and 1.1 units respectively compared with control.

2.2 $CaCl_2$-extractable Pb and Zn

$CaCl_2$-extractable Pb and Zn content in soil were significantly increased with the apply of S (Fig.2), and reached the highest level in the treatment of S_{160} after 64 d which were 4.23 mg/kg and 0.402 mg/kg, 2.7 and 2.0 times as that of the control respectively.

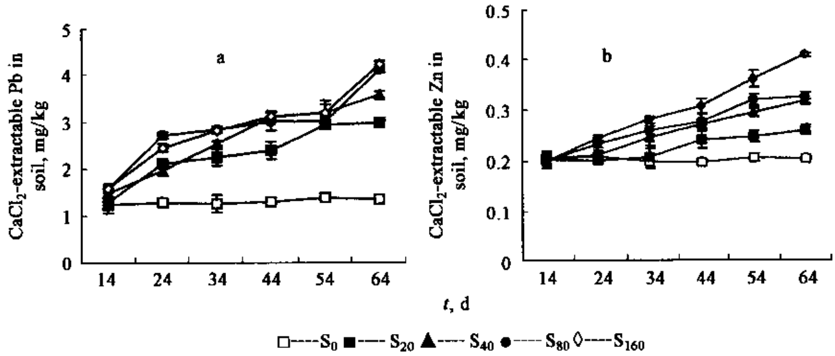

Fig.2 The changes of $CaCl_2$-extractable Pb and Zn contents(mean ± *SE*) in soil at the different S levels

The $CaCl_2$-extractable Pb and Zn contents in soil increased with soil pH decreased. A good correlation between $CaCl_2$-extractable Pb/Zn contents and soil pH was found, R^2 ($n = 25$) were 0.847 and 0.991 respectively(Fig.3).

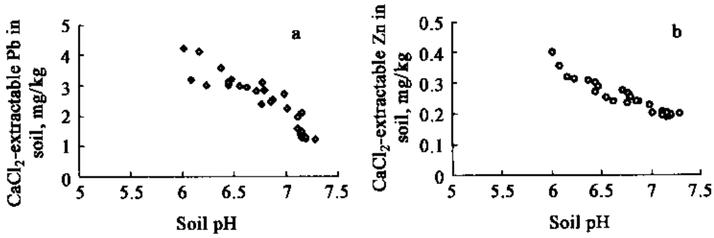

Fig.3 The relation between $CaCl_2$-extractable Pb/Zn contents and soil pH($n = 25$)
a. Pb($R^2 = 0.847$); b. Zn($R^2 = 0.921$)

2.3 Plant growth and metal uptake by plants

Adding S led to a significant yield reduction in Indian mustard and wheat(Table 2), and the toxicity symptoms such as leaf necrosis in all the treatments. Compared to the control, in wheat, the biomass in the treatments of S_{20}, S_{40}, S_{80}, S_{160} had significant decrease, and in Indian mustard, the biomass in treatments S_{40}, S_{80}, S_{160} had significant decrease. In treatment of S_{160}, the biomass of Indian mustard and wheat were 81% and 71% of the controls respectively.

Table 2 Biomass of plants at different S treatments (g/pot) (mean ± *SE*)

Plant	S_0	S_{20}	S_{40}	S_{80}	S_{160}
T. aestivum	$1.31 \pm 0.03^{a*}$	1.14 ± 0.04^{b}	1.10 ± 0.06^{bc}	1.04 ± 0.08^{bc}	0.93 ± 0.02^{c}
B. juncea	0.95 ± 0.04^{a}	0.94 ± 0.03^{ab}	0.86 ± 0.03^{b}	0.81 ± 0.02^{bc}	0.77 ± 0.03^{c}

Notes: * Values followed by the different small letters in the raw are significantly different at the 0.05 levels, according to LSD test

Plants responded to the treatments of S by increased uptake of metals(Table 3). Pb contents in shoots of Indian mustard and winter wheat were all increased with the S application increased, and the significant differences from the controls were all observed in the treatments of S_{40}, S_{80}, and S_{160} in these two crops. The highest Pb content in wheat was 32.8 mg/kg at the treatment S_{160}, 1.8 times as that of the control, and at the same treatment, the highest Pb content in the Indian mustard was 537.0 mg/kg, 1.8 times as that of the control too. Zn contents in shoots of Indian mustard and wheat were all significantly increased in all the treatments compared with the control. In the treatment of S_{160}, the highest Zn content was observed both in the Indian mustard and wheat, they were 404.0 mg/kg and 215.5 mg/kg, 1.4 and 1.0 times greater than that of the controls.

Table 3 Pb and Zn contents in shoots(mg/kg) (mean ± *SE*)

Plant	S_0	S_{20}	S_{40}	S_{80}	S_{160}
Pb *T. aestivum*	17.9 ± 0.7d*	20.1 ± 1.0d	23.5 ± 0.6c	28.2 ± 0.5b	32.8 ± 1.1a
B. juncea	304.8 ± 13.3e	360.1 ± 26.6c	421.6 ± 15.9bc	453.0 ± 20.4b	537.0 ± 27.09a
Zn *T. aestivum*	106.8 ± 4.9d	155.0 ± 3.5c	189.7 ± 5.9b	203.6 ± 4.0ab	215.5 ± 9.9a
B. juncea	171.4 ± 9.7d	196.9 ± 10.1c	230.9 ± 10.8c	326.2 ± 15.7b	404.0 ± 13.0a

Notes: * Values followed by the different small letters in the raw are significantly different at the 0.05 levels, according to LSD test

2.4 Metal removal

Large differences occurred between Indian mustard and wheat in removing Pb, but much less difference between them in removing Zn(Table 4). Pb removal in wheat had a significant increase in the treatment of S_{80} and S_{160}, but significantly differences to the control were observed at the treatment S_{40}, S_{80} and S_{160} in Indian mustard. Zn removal in Indian mustard and wheat had a significantly increase in all the treatments compared with the controls. The highest Pb removal by Indian mustard and wheat were 0.412 g/pot and 0.031 g/pot, 1.4 and 1.3 times as that of the control respectively in the treatment of S_{160}. In the treatment of S_{80}, the highest Zn removal in wheat was observed, but the highest Zn removal in the Indian mustard was observed in the treatment of S_{160}, they were 1.5 and 1.9 times as that of the controls respectively.

Table 4 Pb and Zn removed by plants(g/pot) (mean ± *SE*)

Plant	S_0	S_{20}	S_{40}	S_{80}	S_{160}
Pb *T. aestivum*	0.023 ± 0.001b*	0.023 ± 0.002b	0.026 ± 0.002b	0.029 ± 0.002ab	0.031 ± 0.001a
B. juncea	0.290 ± 0.003b	0.337 ± 0.014b	0.363 ± 0.023ab	0.368 ± 0.024ab	0.412 ± 0.006a
Zn *T. aestivum*	0.140 ± 0.010e	0.177 ± 0.004d	0.209 ± 0.014b	0.213 ± 0.019a	0.201 ± 0.010c
B. juncea	0.164 ± 0.012e	0.185 ± 0.006d	0.198 ± 0.006c	0.265 ± 0.017b	0.311 ± 0.016a

Note: * Values followed by the different small letters in the raw are significantly different at the 0.05 levels, according to LSD test

3 Discussion

In the S treatments, an obvious decrease was observed in soil pH, one important reason might be that certain groups of acidophillic soil bacteria, predominantly the genus *Thiobacillus* in soil, could oxidize S and change soil pH(Lee, 1988; Tichý, 1997; Kayser, 2000). Tichý(Tichý, 1997) reported that adding 156 mmol of different types of elemental sulfur per kg of soil to a gleyic cambisol at an initial soil pH from 5 to 5.5 caused a soil pH decrease down to 3.3 within 80 days. Kayser(Kayser, 2000) reported that adding 36 mol S/m^2 to the soil made soil pH decreased from 7.2 to 6.9. In this experiment, adding S also made the soil pH decreased, the highest decrease was 1.1 units. The results agree well with that of Tichý and Kayser.

There are many methods to extract the mobile metal fraction in the soil. Different extractants may get the difference results. In this experiment, $CaCl_2$ was used to extract mobile metal fraction and the mobile metal fraction greatly influenced by soil pH and generally the extract was increased with decreasing pH. Kayser(Kayser, 2000) used the $NaNO_3$ to extract metals from soil, and found that $NaNO_3$-extractable Cd and Zn increased 35 and 8 times respectively when the soil pH decreased from 7.2 to 6.9. In this experiment, the $CaCl_2$-extractable Pb and Zn increased 1.7 and 1.0 times in the treatment of S_{160} respectively when the pH decreased from 7.11 to 6.01. Whereas Pb is an immobile metal in soils, it had a certain increase with the soil pH decrease also(Basta, 1993; Martínez, 2000). The results agreed with the reported above.

It is well known that Pb is an immobile metal in soils since it readily forms a precipitate within the soil matrix, with a low aqueous solubility, and, in many cases, is not readily biovailiable. In addition, many plants retain Pb in their roots via sorption and precipitation with only minimal transport to the aboveground harvestable plant portions(Kumar, 1995; Huang, 1996; Brennan, 1999). In the same treatment of S, much higher soluble Pb concentration was observed than soluble Zn concentrations. But the proportion of the total contents of Pb is much less than that of Zn. The main reason may be the higher total Pb concentrations in soil, the highly immobile in soils and poor transportation of Pb from the roots to the harvestable shoots.

While application S can enhance metal mobility and increase the uptake into plants, the potential of moving into the groundwater is also increased, producing a detrimental impact on the environment. Therefore, the appropriate S application must be chosen for in agriculture, the appropriate S must be high enough to mobilize the metals in the root zone and not too high to cause toxicity or elevate S concentration in groundwater.

Applying S negatively affected growth of plants, indicating these plants were sensitive to the growing conditions. The reasons might be that adding S made soil pH decreased and increased metal mobility, which changed the growing conditions. The Pb contents in the plants increased less than that of Zn, the main reason was that it had a low solubility and did not transport easily from root to shoot.

It is well known that a good phytoextraction method should have an optimum heavy metal removal. Because the negative effects of S treatments on plant growth, the optimum treatment of S in phytoextraction needs not the result of maximum metal solubility in the soil or maximum metal uptake, but the maximum heavy metal removal. In this experiment, the optimum of S treatments in phytoextract Pb and Zn were all S_{160} by Indian mustard.

4 Conclusions

Soil pH decreased with the elemental sulfur application, metal solubility increased in all treatments, differences in the ratio of solubilized metals versus total metals and differences between solubilized Pb and Zn were observed. The uptake of Pb and Zn by Indian mustard and wheat were all increased with the elemental sulfur amendment. Indian mustard, the Pb and Zn hyperaccumulator, accumulated Pb and Zn in shoot more than that of wheat with or without elemental sulfur amendment. All the results indicated that elemental sulfur amendment may be an effective tool to lower soil pH and subsequently enhance the solubility of Pb and Zn particularly in neutral soil.

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(Received for review August 19, 2002. Accepted September 19, 2002)