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Enhanced uptake of soil Pb and Zn by Indian mustard and winter wheat following combined soil application of elemental sulphur and EDTA

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Abstract

Enhancement of Pb and Zn uptake by Indian mustard (*Brassica juncea* (L.) Czern.) and winter wheat (*Triticum aestivum* L.) grown for 50 days in pots of contaminated soil was studied with application of elemental sulphur (S) and EDTA. Sulphur was added to the soil at 5 rates (0–160 mmol kg⁻¹) before planting, and EDTA was added in solution at 4 rates (0–8 mmol kg⁻¹) after 40 days of plant growth. Additional pots were established with the same rates of S and EDTA but without plants to monitor soil pH and CaCl₂-extractable heavy metals. The highest application rate of S acidified the soil from pH 7.1 to 6.0. Soil extractable Pb and Zn and shoot uptake of Pb and Zn increased as soil pH decreased. Both S and EDTA increased soil extractable Pb and Zn and shoot Pb and Zn uptake. EDTA was more effective than S in increasing soil extractable Pb and Zn, and the two amendments combined had a synergistic effect, raising extractable Pb to >1000 and Zn to >6 times their concentrations in unamended control soil. Wheat had higher shoot yields than Indian mustard and increasing application rates of both S and EDTA reduced the shoot dry matter yields of both plant species to as low as about half those of unamended controls. However, Indian mustard hyperaccumulated Pb in all EDTA treatments tested except the treatment with no S applied, and the maximum shoot Pb concentration was 7100 mg kg⁻¹ under the highest application rates of S and EDTA combined. Wheat showed similar trends, but hyperaccumulation (1095 mg kg⁻¹) occurred only at the highest rates of S and EDTA combined. Similar trends in shoot Zn were found, but with lower concentrations than Pb and far below hyperaccumulation, with maxima of 777 and 480 mg kg⁻¹ in Indian mustard and wheat. Despite their lower yields, Indian mustard shoots extracted more Pb and Zn from the soil (up to 4.1 and 0.45 mg pot⁻¹) than did winter wheat (up to 0.72 and 0.28 mg pot⁻¹), indicating that the effects of S and EDTA on shoot metal concentration were more important than yield effects in determining rates of metal removal over the growth period of 50 days. Phytoextraction of Pb from this highly contaminated soil would require the growth of Indian mustard for nearly 100 years and is therefore impractical.

Introduction

Lead and Zn are two of the most common heavy metal contaminants in the environment. Human activities such as mining, smelting, dumping of municipal

sewage wastes, manufacturing processes and disposal of used batteries are the main sources of environmental contamination. Lead is not an essential element in metabolic processes in plants or animals, and it can accumulate to levels that are toxic or lethal to organisms. Zinc is an essential element in plant and animal metabolic processes, but it can also accumulate to toxic concentrations in the environment (Lock and Janssen,

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2001). There is considerable interest in the development of low-cost techniques for remediation of sites contaminated with Pb and Zn that have minimal environmental side effects (Jung and Thornton, 1997; Martínez and Motto, 2000; Markus and McBratney, 2001).

Phytoremediation (and especially phytoextraction) has recently been proposed as an effective method to remediate soils contaminated with Pb and Zn (Brennan and Shelley, 1999; Bunzl et al., 2001; Lock and Janssen 2001; Salt et al., 1995). Phytoextraction is an environmental friendly and cost-effective approach that uses green plants for the *in situ* risk reduction for contaminated soil, sludge, sediments, and groundwater through contaminant removal. This process has been investigated in several field experiments (Blaylock et al., 1997; Kayser et al., 2000).

There are two main strategies: The use of hyperaccumulator plant species that typically have relatively low yields and use of non-hyperaccumulator species with higher yields but lower metal uptake capacities. The criterion for hyperaccumulation varies for different metals, but was summarized by Baker et al. (1994) as >1000 mg for Pb and $>10\,000$ mg for Zn kg^{-1} of shoot dry matter. The bioavailability of heavy metals is an important factor in the process of phytoextraction by non-hyperaccumulators, especially in neutral or calcareous soils. There are two main approaches that have been used to increase the bioavailability of heavy metals in soils: Lowering soil pH (Blaylock et al., 1997; Chlopecka et al., 1996; Salt et al., 1995) and adding synthetic chelates (Blaylock et al., 1997) such as EDTA, NTA and DTPA. There have been numerous studies focusing on one of these two approaches but few have dealt with the combined use of both approaches (Blaylock et al., 1997; Chlopecka et al., 1996; Kayser et al., 2000; Salt et al., 1995). Ethylenediaminetetraacetic acid (EDTA) is an effective chelate that has been found to increase the solubility of heavy metals in soil and their bioavailability to plants, and the effect can be improved by decreasing soil pH (Blaylock et al., 1997).

A decrease in soil pH can be achieved by application of mineral or organic acids or acid-producing fertilizers such as ammonium chloride (Huang et al., 1997; Salt et al., 1995; Wasay et al., 1998). However, these methods have some limitations due to negative effects on soil fertility or soil structure, or they may lead to groundwater pollution. To avoid some of these constraints, the use of elemental S has been suggested to decrease soil pH and increase solubility of heavy

metals in soils (Kayser et al., 2000; Tichý et al., 1997). Certain groups of acidophilic soil bacteria, predominantly the genus *Thiobacillus*, can metabolise S in the presence of O_2 and generate H^+ and SO_4^{2-} , leading to soil acidification (Kayser et al., 2000; Lee et al., 1988; Tichý et al., 1997). Thus, first lowering soil pH and then adding EDTA might be an effective strategy to maximize the phytoextraction of Pb and Zn from contaminated soils that are mostly neutral or slightly alkaline. A pot experiment was therefore designed to test this strategy. Elemental S and EDTA were added at different rates to Pb- and Zn-contaminated soil in which Indian mustard and winter wheat were grown. The main objectives of the study were to investigate the influence of S application on soil acidification and metal solubility and the effectiveness of EDTA and S applied alone or together on soil metal solubility and uptake by plants.

Materials and methods

Soil

The top 20 cm of soil was collected from a profile near a battery-recycling factory in Anhui province, China. The soil was air-dried, ground and sieved by passing through a 2-mm sieve. Physicochemical properties of the soil were as follows: pH (in 0.01 M CaCl_2) 7.1, CEC 6.05 cmol kg^{-1} , clay 25.1%, silt 42.8%, sand 32.1%, total Pb 31 000 mg kg^{-1} , total Zn 480 mg kg^{-1} , 0.01 M CaCl_2 -extractable Pb 1.59 mg kg^{-1} , and CaCl_2 -extractable Zn 0.20 mg kg^{-1} .

Experimental design and treatments

An orthogonal experimental design was employed with elemental S applied at 5 rates (0, 20, 40, 80 and 160 mmol kg^{-1}) before planting and EDTA disodium salt at 4 rates (0, 2, 4, 8 mmol kg^{-1} soil) 40 days after planting. Fertilizer was applied at rates of 120 mg N (NH_4NO_3), 80 mg P (KH_2PO_4) and 120 mg K kg^{-1} (KCl and KH_2PO_4) with N:P:K = 1:0.67:1. Sulphur and fertilizer were thoroughly mixed prior to potting. Each pot received 500 g of soil, which was allowed to equilibrate for a period of 14 days before the plant seeds were sown in the glasshouse. Indian mustard (*Brassica juncea* (L.) Czern.) and winter wheat (*Triticum aestivum* L.) seeds were sown at a rate of 15 pot^{-1} and thinned to leave 8 seedlings in each pot one week after germination. The experiment was

carried out in a glasshouse with an artificial irradiance of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density over a manually-controlled 14-h photoperiod each day. Soil water content was adjusted regularly by the weight equivalent to about 60% of water holding capacity. Plants were grown for 50 days. Corresponding rates of EDTA were applied to the soil in aqueous solution (25 ml per pot) 10 days before the plants were harvested. There were three replicates of each treatment and additional pots were set up with the same levels of S and EDTA but without plants, which were used for soil sampling to monitor changes in soil pH and CaCl_2 -extractable heavy metals during the course of the experiment.

Sampling and analysis

Soil samples were collected from the pots with no plants at 10-day intervals throughout the experiment using a cylindrical soil sampler. The samples were passed through a 2-mm nylon sieve. Soil pH was measured on sub-samples using a 1:2.5 soil: 0.01 M CaCl_2 ratio by pH meter. Twenty mL of 0.01 M CaCl_2 were added to 2.00 g of air-dried soil in a 50-mL polypropylene centrifuge tube. The tube was shaken end-over-end for 16 h at 25°C , then centrifuged at 4000 rpm for 10 min. The supernatant was decanted for Pb and Zn determination by atomic absorption spectrophotometry (AAS) or inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Novozamsky et al., 1993). Sub-samples (0.1000 g) were digested with a mixture of concentrated $\text{HNO}_3\text{:HClO}_4\text{:HF}$ (3:1:1 in volume) and soil total Pb and Zn were determined by AAS. The harvested plant samples were rinsed with deionized water, oven dried at 70°C for 48 h, ground with an agate mill, and sub-samples (0.1000 g) were digested and then analyzed as described above for the soil samples.

Statistical analysis

All data were subjected to two-way or three-way analysis of variance (ANOVA), and correlation analysis was used to compare soil pH and CaCl_2 -extractable Pb and Zn concentrations using the SAS package (version 8.1, SAS Inc., Cary, NC, USA).

Figure 1. Changes in soil pH (mean $\pm$ SE) under different rates of S application in pots with no plants throughout the growth period of the plants. $\square$ 0, $\blacksquare$ 20, $\blacktriangle$ 40, $\bullet$ 80, and $\diamond$ 160 mmol kg^{-1} applied S.

Figure 2. Changes in soil CaCl_2 -extractable (a) Pb and (b) Zn (mean $\pm$ SE) under different rates of S application throughout the growth period of the plants. $\square$ 0, $\blacksquare$ 20, $\blacktriangle$ 40, $\bullet$ 80, and $\diamond$ 160 mmol kg^{-1} applied S.

Figure 3. Relationship between CaCl_2 -extractable (a) Pb ($R^2 = 0.847$, $n = 25$) and (b) Zn ($R^2 = 0.921$, $n = 25$) and soil pH.

Results

Soil pH

Application of S decreased soil pH and the highest application rate of S acidified the soil pH from 7.11 to 6.00. (Figure 1).

Soil extractable Pb and Zn as influenced by application of S

Soil CaCl_2 -extractable Pb and Zn concentrations were significantly increased by the application of S (Figure 2). The highest concentrations were 4.2 mg Pb and 0.40 mg Zn kg^{-1} , 2.7- and 2.0-fold higher than the control for 160 mmol S kg^{-1} . Soil extractable Pb and Zn concentrations increased as soil pH decreased, and good negative correlations were found between CaCl_2 -extractable Pb or Zn and soil pH, with R^2 values of 0.847 and 0.991, respectively (Figure 3).

Soil extractable Pb and Zn with EDTA amendment

EDTA increased soil extractable Pb and Zn concentrations compared to the control with or without applica-

Table 1. Soil CaCl_2 -extractable Pb and Zn as influenced by application of S and EDTA in the absence of crop plants

EDTA rate (mmol kg ⁻¹)	S rate (mmol kg ⁻¹)					
	0	20	40	80	160	Mean
Pb (mg kg⁻¹)						
0	1	3	4	4	4	3
2	363	430	912	1009	741	691
4	688	1090	1108	1102	859	970
8	821	1114	1123	1148	922	1026
Mean	468	659	787	816	632	672
Significance ¹						
Sulphur (S)			***			
EDTA			***			
S × EDTA			***			
Zn (mg kg⁻¹)						
0	0.199	0.256	0.311	0.321	0.402	0.298
2	0.241	0.298	0.503	0.592	0.594	0.446
4	0.405	0.499	0.602	1.107	0.998	0.722
8	0.526	1.001	1.312	1.009	1.005	0.971
Mean	0.343	0.514	0.682	0.757	0.750	0.609
Significance						
Sulphur (S)			***			
EDTA			***			
S × EDTA			***			

¹By analysis of variance; ***, $P < 0.001$.

tion of S, and the concentrations of soil extractable Pb and Zn also increased with application of S up to a rate of 80 mmol kg^{-1} (Table 1). On average, application of EDTA at 8 mmol kg^{-1} increased soil extractable Pb by over 1000 mg kg^{-1} compared with 3 mg kg^{-1} , and Zn by 0.9 mg kg^{-1} compared with 0.3 mg kg^{-1} in the control (without EDTA). Similarly, application of S at a rate up to 80 mmol kg^{-1} increased soil extractable Pb from 468 to 816 mg kg^{-1} , and Zn from 0.3 to 0.8 mg kg^{-1} . Additional S (160 mmol kg^{-1}) gave a decrease in soil extractable Pb and no effect on Zn (Table 1). EDTA alone was more effective than S alone in elevating soil extractable Pb and Zn. The combination of 80 mmol S and 8 mmol EDTA kg^{-1} soil gave the highest extractable Pb of 1148 mg kg^{-1} representing >1000 times the unamended control value, and 40 mmol S together with 8 mmol EDTA gave 1.3 mg extractable Zn kg^{-1} , almost 7 times the control value.

Shoot yields

Indian mustard had lower shoot yields than winter wheat, and shoot yields of both plant species declined with increasing application of S and EDTA (Table 2). In the treatment with the highest additions of S and EDTA, the shoot yield of Indian mustard declined to 60% of the unamended control and the yield of winter

Table 2. Shoot dry matter yields of Indian mustard and winter wheat as influenced by application of S and EDTA

EDTA rate (mmol kg ⁻¹)	S rate (mmol kg ⁻¹)					Mean
	0	20	40	80	160	
Indian mustard (g pot ⁻¹)						
0	0.953	0.940	0.860	0.810	0.770	0.867
2	0.920	0.790	0.783	0.713	0.650	0.771
4	0.827	0.727	0.707	0.680	0.637	0.715
8	0.807	0.730	0.677	0.613	0.580	0.681
Mean	0.877	0.797	0.757	0.704	0.659	0.759
Winter wheat (g pot ⁻¹)						
0	1.307	1.143	1.100	1.043	0.933	1.105
2	1.260	1.147	1.080	1.053	0.913	1.091
4	1.033	0.970	0.867	0.847	0.780	0.899
8	0.890	0.843	0.780	0.717	0.657	0.777
Mean	1.122	1.026	0.957	0.915	0.821	0.968
Significance ¹						
Crop species				***		
Sulphur (S)				***		
EDTA				***		
Crop × S				NS		
Crop × EDTA				***		
S × EDTA				NS		

¹By analysis of variance; ***, $P < 0.001$; NS, not significant.

wheat declined to 50% of the control (Table 2). The pattern of the results and the two-way interactions in the ANOVA indicate that S had similar effects on both plant species but EDTA had a stronger yield effect on winter wheat than on Indian mustard. The yield effects of S and EDTA were not interactive.

Shoot Pb and Zn concentrations

Indian mustard had higher shoot metal concentrations than wheat, especially of Pb, and both S and EDTA increased the shoot metal concentrations of in both plant species (Table 3). EDTA alone was more effective than S alone and the two amendments combined gave an interaction which produced a synergistic effect, leading to hyperaccumulation of Pb in Indian mustard in all of the EDTA treatments except the lowest rate with no S application. Maximum Pb concentrations occurred when the highest rate of both amendments was applied, reaching 7100 and 1095 mg kg⁻¹ in Indian mustard and winter wheat. Shoot Zn concentrations followed similar trends to Pb but were approached hyperaccumulation in either plant species.

Shoot Pb and Zn uptake

Indian mustard shoots took up more Pb and Zn than did winter wheat (Table 4). Both S and EDTA in-

Table 3. Shoot Pb and Zn concentrations in winter wheat and Indian mustard as influenced by application of S and EDTA

EDTA rate (mmol kg ⁻¹)	S rate (mmol kg ⁻¹)					Mean	
	0	20	40	80	160		
Pb (mg kg⁻¹)							
Indian mustard							
0	305	360	422	453	537	415	
2	952	1055	1141	1469	1600	1243	
4	2041	2504	3286	5120	6228	3836	
8	3533	3775	4180	5983	7100	4914	
Mean	1708	1923	2257	3256	3866	2602	
Winter wheat							
0	18	20	24	28	33	24	
2	165	164	183	258	307	215	
4	321	355	387	435	458	391	
8	479	547	846	981	1095	790	
Mean	246	272	360	425	473	355	
Significance ¹							
Crop species			***				
Sulphur (S)			***				
EDTA			***				
Crop × S			***				
Crop × EDTA			***				
S × EDTA			***				
Zn (mg kg⁻¹)							
Indian mustard							
0	171	197	231	326	404	266	
2	351	427	474	502	546	460	
4	384	447	512	608	650	520	
8	501	569	595	679	777	624	
Mean	352	410	453	529	594	468	
Winter wheat							
0	107	155	190	204	216	174	
2	198	251	257	271	307	257	
4	253	297	311	366	400	326	
8	327	383	415	446	480	410	
Mean	221	272	293	322	351	292	
Significance ¹							
Crop species			***				
Sulphur (S)			***				
EDTA			***				
Crop × S			***				
Crop × EDTA			***				
S × EDTA			**				

¹By analysis of variance; ***, $P < 0.001$; **, $P < 0.01$.

creased metal removal by the shoots and EDTA alone was more effective than S alone. The highest uptake values occurred at the highest rates of both amendments combined except for Zn uptake by winter wheat which reached maximum values with 8 mmol EDTA kg⁻¹ and 20–40 mmol S kg⁻¹. Maximum quantities of Pb removed by Indian mustard and wheat were 4.12 and 0.45 mg pot⁻¹ and of Zn were 0.72 and 0.32 mg pot⁻¹. Application of S and EDTA together had a synergistic effect on shoot uptake of the metals.

Table 4. Shoot Pb and Zn uptake by Indian mustard and winter wheat as influenced by application of S and EDTA

EDTA rate (mmol kg ⁻¹)	0	S rate (mmol kg ⁻¹)				Mean
		20	40	80	160	
Pb (mg pot ⁻¹)						
Indian mustard						
0	0.290	0.337	0.363	0.368	0.412	0.354
2	0.877	0.834	0.895	1.050	1.037	0.939
4	1.689	1.822	2.321	3.483	3.958	2.654
8	2.844	2.753	2.831	3.669	4.122	3.244
Mean	1.425	1.437	1.602	2.142	2.382	1.798
Winter wheat						
0	0.023	0.023	0.026	0.029	0.031	0.026
2	0.208	0.188	0.197	0.271	0.280	0.229
4	0.332	0.345	0.336	0.367	0.358	0.348
8	0.426	0.461	0.660	0.702	0.723	0.594
Mean	0.247	0.254	0.305	0.343	0.348	0.299
Significance ¹						
Crop species			***			
Sulphur (S)			***			
EDTA			***			
Crop × S			***			
Crop × EDTA			***			
S × EDTA			***			
Zn (mg pot ⁻¹)						
Indian mustard						
0	0.164	0.185	0.198	0.264	0.311	0.224
2	0.322	0.338	0.371	0.358	0.356	0.349
4	0.317	0.325	0.362	0.413	0.414	0.366
8	0.404	0.416	0.403	0.416	0.451	0.418
Mean	0.302	0.316	0.333	0.363	0.383	0.339
Winter wheat						
0	0.140	0.177	0.209	0.213	0.201	0.188
2	0.249	0.287	0.276	0.285	0.280	0.276
4	0.262	0.289	0.270	0.310	0.311	0.288
8	0.290	0.322	0.324	0.320	0.314	0.314
Mean	0.235	0.269	0.270	0.282	0.276	0.266
Significance ¹						
Crop species			***			
Sulphur (S)			***			
EDTA			***			
Crop × S			***			
Crop × EDTA			***			
S × EDTA			**			

¹By analysis of variance; ***, $P < 0.001$; **, $P < 0.01$.

Discussion

This study compared removal of soil Pb and Zn using two non-hyperaccumulator plants, one of which (Indian mustard) had lower yields but hyperaccumulated Pb in the shoots after application of synthetic chelate to the soil and accumulated >7000 mg kg at the highest rates of EDTA and S tested. The higher-yielding cereal also hyperaccumulated Pb, but only when both amendments were applied at the highest rates tested and only to a shoot concentration of 1095

mg kg⁻¹. Lead has very limited solubility in soils and thus availability for plant uptake due to complexation with soil organic matter, sorption on oxides and clay minerals and precipitation as carbonates, hydroxides and phosphates (McBride, 1994). Enhancement of Pb accumulation by Indian mustard from soils amended with a range of chelates was reported by Blaylock et al. (1997), who found that EDTA was the most effective chelate tested and that citric acid was not effective. Römken et al. (2002) have attributed the limited effectiveness of citric acid to its microbial degradation within a few days of application to the soil. In contrast, metal lability can persist for several weeks after application of EDTA to soils, and this may represent a risk of groundwater contamination (Puschenreiter et al., 2001).

The mobile fraction of soil heavy metals is greatly influenced by soil pH and generally increases as soil pH decreases. Neutral unbuffered salt solutions such as CaCl₂ and NaNO₃ are often used as extractants in single extraction procedures for determination of heavy metals in contaminated soil (Rauret, 1998). Kayser et al. (2000) used NaNO₃ to extract metals from soil, and found that NaNO₃-extractable Cd and Zn increased 35- and 8-fold, respectively, when soil pH was lowered from 7.2 to 6.9. In our experiment, S application at 160 mmol S kg⁻¹ increased the concentrations of soil CaCl₂-extractable Pb and Zn by 2.7- and 2.0-fold at most compared to the controls, resulting in a decrease in soil pH from 7.11 to 6.01. Although Pb is a relatively immobile metal in soils as discussed above, the extractable concentration can show some increase with a decrease in soil pH (Basta et al., 1993; Martínez and Motto, 2000). However, the lower effectiveness of S application at 160 mmol kg⁻¹ for Pb compared with Zn may be attributable to the greater effect of soil pH on solubility of Zn which is less affected by organic matter. Alternatively, the observation that the highest rate of S application lowered the concentration of soil CaCl₂-extractable Pb but increased shoot Pb concentrations may indicate that this fraction of soil Pb may be smaller than the fraction actually available to plants. Further studies would be required to determine the best extractant for accurate measurement of the fraction of soil Pb that is available to plants.

Although soil amendment with EDTA was more effective than application of S, the combined application of both agents had a synergistic effect on the extractability of Pb and Zn. Yu and Klarup (1994) studied the influence of pH and concentration of

EDTA on the solubilisation of various metals in contaminated sediments and found that in all cases studied the lower the pH and the higher the EDTA concentration, the greater the extraction recovery. Puschenreiter et al. (2001) found that EDTA was much more effective than $(\text{NH}_4)_2\text{SO}_4$ at increasing the concentration of soil NH_4NO_3 -extractable Pb.

Shoot yields of both plant species declined with application of S and EDTA, indicating the possible occurrence of direct toxicity of EDTA or indirect toxicity via increased metal solubility. Decreasing yields of plant shoots with application of EDTA has been reported in several studies (Blaylock et al., 1997; Chen and Cutright, 2001). When S was applied alone, Kayser et al. (2000) reported that shoot weight in some plants did not change significantly. However, in the present experiment, application of S decreased the yield of the plant shoots, and this may have been due to phytotoxicity from the increase in solubility and bioavailability of the heavy metals.

In addition to its low geochemical mobility and thus low bioavailability in most soils, Pb is retained by many plants in their roots via sorption and precipitation, with only minimal transport to the aboveground harvestable plant portions (Brennan and Shelley, 1999; Huang and Cunningham, 1996; Kumar et al., 1995). However, in the present study, application of EDTA and S led to substantial increases in shoot Pb concentrations. These effects were so marked that shoot uptake of Pb and Zn was greatly enhanced despite the adverse effects of the amendments on shoot yields.

Despite the significant effects of the amendments on shoot metal uptake (especially uptake of Pb by Indian mustard), phytoextraction would not be a successful strategy for the remediation of Pb in this heavily polluted soil. The soil contains >3% total Pb, and even with a shoot concentration of 1% and an average yield of 30 tonnes ha^{-1} , it would take nearly 100 years to bring the Pb concentration down to the Chinese limit for soils at pH 6.5–7.5 of 300 mg kg^{-1} (Chinese National Standard GB15618-1995) in the top 10 cm. Thus, even with chelate and S enhancement combined, phytoextraction of Pb would only be a practical proposition for lightly or moderately polluted soils.

Conclusions

Both amendments increased the extractable fractions of soil Pb and Zn. EDTA was more effective than S.

Shoot uptake of Pb and Zn by Indian mustard and wheat was increased with S and EDTA amendments. Indian mustard shoots took up more Pb and Zn than winter wheat with or without S and EDTA amendment. The optimum treatment for accumulation of Pb and Zn by winter wheat and Indian mustard was application of S combined with EDTA. Combined application of S and EDTA can be an effective approach to lower soil pH and increase the bioavailability and plant uptake of Pb and Zn. Combined chemical enhancement could be used for the phytoremediation of soils lightly contaminated with Pb but not of the heavily contaminated soil used in this study.

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