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Effect of Elemental Sulphur on Uptake of Cadmium, Zinc, and Sulphur by Oilseed Rape Growing in Soil Contaminated with Zinc and Cadmium

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ABSTRACT

A pot experiment was conducted to study the influence of elemental sulphur (S⁰) on cadmium (Cd), zinc (Zn), and sulphur (S) uptake by oilseed rape (*Brassica napus* L.) grown in a calcareous soil contaminated with either Cd or Zn or with both Zn and Cd together. Elemental sulphur was applied at two rates (0 and 200 mmol), Cd at two rates (0 and 20 mg), and Zn at three rates (0, 20, and 200 mg) kg⁻¹ soil. Cadmium (as CdCl₂) and Zn (as ZnCl₂)

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were added in solution to soil prior to planting. After 10 days, S^0 and fertilizer were thoroughly mixed and added to the soil. Plants were harvested after growing for 40 days in a greenhouse. Soil pH and shoot dry weight decreased significantly in response to S^0 application. Addition of Zn and Cd did not influence plant growth without S^0 supply. Shoot concentrations of Zn and Cd increased significantly with Zn and Cd addition as well as S^0 supply. Cadmium addition did not lead to any change in shoot Zn concentration. Shoot Cd concentration decreased in the 200 mg kg^{-1} Zn addition treatment compared with the control but remained unchanged in all Zn treatments when S^0 was applied. Shoot S concentration increased with application of S^0 , Zn, and Cd. Shoot Zn and Cd uptake by oilseed rape increased significantly with the Zn and Cd addition. However, shoot Zn removal did not increase significantly, and Cd removal decreased when S was applied. In conclusion, S^0 acidified the soil and increased shoot concentrations of Zn and Cd, but its depressive effect on shoot biomass was so pronounced that it would not enhance phytoextraction of Cd or Zn from this soil by oilseed rape.

Key Words: Bioavailability; Heavy metals; Phytoremediation; Shoot uptake; Soil acidification.

INTRODUCTION

Soils can be contaminated with Cd and Zn from sewage sludge or urban composts, fertilizers, emissions from municipal-waste incinerators, residues from metal smelting and refining, and other human activities. Cadmium is a nonessential element for plants and is generally considered as a toxic contaminant in food for humans and animal feeds.^[1] Zinc is an essential nutrient for plant growth, but concentrations of Zn in contaminated soils have frequently been found to exceed plant requirements and may cause phytotoxicity.^[2] Excessive accumulation of Cd and Zn in soils must therefore be avoided or remediated to avoid their transfer to plants and food chains.^[3]

There is currently no suitable method for removal of Cd and Zn from soils in the short term while preserving soil properties. Phytoextraction is one option for remediation of metal-contaminated soils, and the bioavailability of heavy metals is an important factor in the process of phytoextraction.^[2-4]

There are many soil properties that influence the bioavailability of soil Cd and Zn, including pH, types of minerals present, types and amounts of anions in the soil solution, organic-matter content, and Zn or

Cd carriers.^[5–7] Soil pH is often one of the most important properties influencing the mobility and bioavailability of Cd and Zn in soils.^[6,7] A decrease in soil pH can be achieved by application of mineral or organic acids or acidifying fertilizers such as ammonium chloride.^[8–10] However, these methods have limitations due to their negative effects on soil's chemical, physical, and biological properties. The use of S⁰ has therefore been suggested for alleviation of these constraints and for soil acidification to increase the solubility of metals in contaminated soils.^[11–13] However, there have been few studies that have evaluated the feasibility of this method as a tool for enhancement of metal uptake by plants growing in soils with different levels of Cd and Zn in addition to plant uptake of S. The objectives of our study were to investigate the tissue concentrations and uptake rates of Cd, Zn, and S in oilseed rape that was grown in soil experimentally contaminated with different levels of Cd and Zn with and without application of S⁰.

MATERIALS AND METHODS

Soil

Soil was collected from the top 20 cm of the profile in a field in Qinghe, Beijing, China. The soil was air-dried, ground, passed through a 2-mm sieve and had the following physico-chemical properties prior to the experiment: pH (in 0.01 M CaCl₂) 7.25, organic-matter content 12 g, total Zn 68 mg, and total Cd 0.8 mg kg⁻¹.

Plant Cultivation

Oilseed rape (*Brassica napus* L. cv. Siyueman) seeds were sown at a rate of 6 pot⁻¹ and thinned to three seedlings in each pot 1 week after germination. The experiment was carried out in a glasshouse with a 14 h (28°C)/10 h (15°C) day/night cycle. Soil-moisture content was adjusted regularly by weight to about 60% of water-holding capacity and the plants were harvested after 40 days of growth.

Experimental Design and Treatments

The experimental treatments consisted of S⁰ applied at two rates (0 and 200 mmol kg⁻¹), Cd at two rates (0 and 20 mg kg⁻¹ soil), and Zn at

three rates (0, 20, 200 mg kg⁻¹ soil) in a multifactorial design. Thus there were 12 treatments in triplicate, giving a total of 36 pots. Fertilizer was applied at rates of 120 mg N (as NH₄NO₃), 80 mg P (as KH₂PO₄), and 120 mg K kg⁻¹ (as KCl and KH₂PO₄). Cadmium (as CdCl₂) and Zn (as ZnCl₂) were added in solution to portions of soil prior to potting, and 10 days later, elemental sulphur (S⁰) and the fertilizers were thoroughly mixed and added to the soil. Each pot contained 1 kg of soil, which was allowed to equilibrate for a period of 14 days in the glasshouse before the seeds were sown.

Soil and Plant Analysis

Soil samples were taken before the experiment and after the plants were harvested. Soil pH was measured on subsamples using a 1:2.5 soil:0.01 M CaCl₂ ratio using a pH meter. Total Zn and Cd were determined using aliquots of air-dried soil ground to <0.25 mm and digested with a mixture of concentrated HNO₃:HClO₄:HF (3:1:1 v/v/v), and total Zn and Cd were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a Perkin-Elmer Optima 2000 spectrophotometer. Plant shoots were harvested and rinsed with deionized water, oven-dried at 70°C for 48 h, ground with an agate mill, digested with a mixture of concentrated HNO₃:HClO₄ (3:1 v/v); total Zn, Cd, and S were analyzed by ICP-OES.

Statistical Analysis

Data were subjected to three-way ANOVA using the SAS package (version 8.1, SAS, Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Soil pH

Soil pH decreased significantly in all S⁰ application treatments with the largest decrease occurring at a combined addition of 200 mmol S, 20 mg Zn, and 20 mg Cd kg⁻¹. Furthermore, the acidifying effect of S⁰ on the soil was more pronounced when Cd or Zn was applied (Table 1). There were significant interactions between S⁰ and Zn and between S⁰ and Cd, but not between Zn and Cd. The soil acidifying effect of S⁰ may

Table 1. Changes in soil pH after plant harvest in soil amended with S⁰, Cd, and Zn.

Zn rate (mg kg ⁻¹)	S ⁰ rate (mmol kg ⁻¹)			
	0		200	
	Cd rate (mg kg ⁻¹)			
	0	20	0	20
0	7.32	7.37	6.39	5.72
20	7.38	7.25	5.53	5.06
200	7.34	7.26	5.41	5.13
Significance ^a				
S ⁰ rate			b	
Zn rate			b	
Cd rate			b	
S ⁰ × Zn			b	
S ⁰ × Cd			c	
Zn × Cd			ns	
S ⁰ × Zn × Cd			ns	

^aBy analysis of variance.^b $p < 0.001$.^c $p < 0.01$.

ns = not significant.

be explained by the activities of certain groups of acidophilic soil bacteria, particularly the genus *Thiobacillus*, which can metabolize S⁰ in the presence of O₂ and generate H⁺ and SO₄²⁻, leading to soil acidification.^[11–14]

Plant Growth

Shoot dry weights decreased with S⁰ application (Table 2). Addition of Zn or Cd did not influence the shoot biomass when S⁰ was not applied. The significant interaction between S⁰ and Zn indicates the smaller shoot biomass at the low Zn application rate in the absence of added S.

Kayser et al.^[11] reported that shoot weight in some plants did not change significantly with S⁰ application. However, in the present study, acidification of the calcareous soil may have depressed the growth of the oilseed rape.

Table 2. Shoot biomass of oilseed rape (g pot^{-1}) grown in soil amended with S^0 , Cd, and Zn.

Zn rate (mg kg ⁻¹)	S ⁰ rate (mmol kg ⁻¹)			
	0		200	
	Cd rate (mg kg ⁻¹)			
	0	20	0	20
0	2.56	2.37	0.66	0.68
20	2.16	2.28	0.82	0.59
200	2.71	2.42	0.42	0.30
Significance ^a				
S ⁰ rate			b	
Zn rate			ns	
Cd rate			ns	
S ⁰ × Zn			c	
S ⁰ × Cd			ns	
Zn × Cd			ns	
S ⁰ × Zn × Cd			ns	

^aBy analysis of variance.^b $p < 0.001$.^c $p < 0.01$.

ns = not significant.

Shoot Concentrations of Zinc and Cadmium

The concentration of Zn in oilseed rape shoots increased with increasing Zn addition and with S^0 application but was unaffected by added Cd (Table 3). Shoot Cd increased significantly with Cd addition and S^0 supply but decreased when 200 mg kg^{-1} Zn was applied in the absence of added S^0 compared with the control. However, when S^0 was applied, the shoot concentration of Cd was unaffected by both application rates of Zn.

Other studies have also found that increasing concentrations of heavy metals in soils led to higher accumulation of heavy metals in plant tissues.^[15,16] Without added S^0 , increasing Zn application rate led to a decrease in shoot Cd concentration, a result that agrees with other studies that have indicated that Zn application can lower Cd concentrations in some plant species.^[17,18] In contrast, as far as we know, there is no published information on the effects of increasing levels of Zn application on Cd concentrations in plants with S^0 application to the soil. In the

Table 3. Shoot Zn, Cd, and S concentrations in oilseed rape grown in soil amended with S⁰, Cd, and Zn.

S ⁰ rate (mmol kg ⁻¹)													
	0			200			0			200			
Cd rate (mg kg ⁻¹)													
	0			20			0			20			
Zn rate (mg kg ⁻¹)	0	20	0	20	0	20	0	20	0	20	0	20	
	Zn (mg kg ⁻¹)						Cd (mg kg ⁻¹)						S (g kg ⁻¹)
0	31.1	39.2	45.1	42.3	ND ^a	66.9	ND	147.0	4.62	5.53	10.90	11.35	
20	103.0	57.4	146.3	102.6	ND	69.6	ND	145.8	5.68	6.11	12.13	12.46	
200	146.6	137.4	1,044.0	1,470.9	ND	45.1	ND	152.1	4.87	5.77	15.17	18.41	
Significance ^b													
S ⁰ rate			c			c					c		
Zn rate			c			ns					c		
Cd rate			ns			ns					ns		
S ⁰ × Zn			c			ns					c		
S ⁰ × Cd			ns			ns							
Zn × Cd			ns			ns							
S ⁰ × Zn × Cd			ns			ns							

^aND = not determined—below detection limit.

^bBy analysis of variance.

 $p < 0.001$.

$p < 0.001$.
ns = not significant.

present study, the shoot Cd concentration did not change significantly under different levels of Zn application with added S^0 . Soil application of S^0 led to increased shoot concentrations of Cd and Zn. This may have been due partly to an increase in the solubility of soil Zn and Cd and an increase in their bioavailability due to soil acidification by S. McLaughlin et al.^[16] reported that increasing SO_4^{2-} in soil increased shoot Cd concentrations. A similar increase in soil SO_4^{2-} may have resulted from application of S^0 in the present experiment. Addition of S^0 to contaminated soils has been reported to increase concentrations of heavy metals and S in plants.^[11,13] In the present study, S application increased shoot concentrations of Zn and Cd in all treatments spiked with Zn or Cd, and this agrees well with the results of Kayser et al.^[11] and Tichý et al.^[13] The other factor that can affect shoot concentrations of the metals is shoot biomass, this can be taken into account by calculating the quantities of the metals taken up by the shoots.

Shoot Sulphur Concentrations

As expected, shoot S concentrations responded to S^0 application, probably due to increased uptake of SO_4^{2-} from the soil and also increased with Zn and Cd addition (Table 3). Using hydroponic culture, Coolong and Randle^[1] found that total S in field mustard (*Brassica rapa*) shoots was higher with Zn supply than in controls without Zn supply when the plants were exposed to Zn levels ranging from 1 to 25 mg L⁻¹. In the present study, the shoot concentrations of S increased significantly with Zn addition whether or not S^0 was applied. This result conflicts with Kumar and Singh^[15] who found that total S in soybean decreased significantly with increasing Zn levels from 5 to 10 mg kg⁻¹. The effects of Zn or Cd supply on total S in plants may depend on numerous factors, such as Zn, Cd, S, and fertilizer sources, crop-growth conditions, and plant species.

Shoot Uptake of Zinc and Cadmium

Shoot Zn and Cd uptake increased significantly with Zn and Cd addition (Table 4). However, S^0 application had no effect on shoot Zn uptake, and it depressed shoot Cd uptake despite the increases in shoot concentrations of the metals reported above. The drastic reduction in shoot biomass produced by S^0 application has therefore prevented the S treatment from increasing the rate of removal of Cd or Zn from the soil by phytoextraction using oilseed rape. Other studies^[19] in which both

Table 4. Shoot Zn and Cd uptake ($\mu\text{g pot}^{-1}$) in oilseed rape grown in soil amended with S^0 , Cd, and Zn.

	S ⁰ rate (mmol kg ⁻¹)					
	0		200		0	
	Cd rate (mg kg ⁻¹)					
Zn rate (mg kg ⁻¹)	0	20	0	20	0	20
	Zn (mg kg ⁻¹)			Cd (mg kg ⁻¹)		
0	79.4	92.2	29.8	28.9	ND ^a	ND
20	221.9	131.6	110.2	61.9	ND	ND
200	400.6	330.7	419.1	405.8	ND	ND
Significance ^b						
S ⁰ rate	ns			c		
Zn rate	c			ns		
Cd rate	ns			ns		
S ⁰ × Zn	ns			ns		
S ⁰ × Cd	ns			ns		
Zn × Cd	ns			ns		
S ⁰ × Zn × Cd	ns			ns		

^aND = mean not determined (see Table 3).

^bBy analysis of variance

^c $p < 0.001$.

ns = not significant.

EDTA and S have been applied to the soil have indicated that the EDTA was more effective than S in mobilizing lead (Pb) and Zn for extraction by Indian mustard and wheat, but S made a useful contribution to metal removal by acting synergistically with the EDTA. Thus it seems that S application on its own may not be effective, but perhaps it can be used in combination with a chelating agent to increase soil-metal bioavailability for phytoextraction.

CONCLUSIONS

Elemental S decreased soil pH and increased shoot concentrations of Zn and Cd of oilseed rape but did not significantly enhance the uptake of the metals. Shoot concentrations of S increased with increasing supply of elemental S, Cd, and Zn. The results indicate that adding elemental S to soil can increase Zn and Cd concentrations, but shoot uptake of the metals may not increase because of a marked depression in shoot biomass. Application of S⁰ alone may therefore be ineffective for enhancement of phytoremediation of calcareous soils by oilseed rape.

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