Journal of Environmental Sciences Vol. 7, No. 4, pp. 399—405, 1995 ISSN 1001—0742

Effect of organic acid on Cd toxicity in tomato and bean growth

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Abstract—A Hoagland's 1 nutrient solution culture to observe the effect of 0, 50 and 100 μmol/L oxalate acid on 0 to 10 mg/L Cd toxicity for tomato and 0 to 8 mg/L for bean and their accumulation in plants were studied. There was an almost linear relationship between Cd in roots and shoots of tomato and bean vs. Cd in the nutrient solution. Tomato roots accumulated about 15 times Cd than the plants' shoots and bean roots 4 times. Root and shoot Cd accumulation vary greatly between plant species. Results also suggest organic acid in the solution can complex Cd, detoxifying the solution. Complexed Cd can be absorbed by roots and transported to shoot and appears to be less toxic. Cd toxicity was reduced, though not completely in the tomato shoots when oxalic acid was added to solutions containing 2.5 mg/L and 5 mg/L Cd. Cadmium content in the shoots still increased. At 2 and 4 mg/L Cd levels, with 25 μmol/L and 50 μmol/L oxalic acid addition in nutrient solution, bean shoot growth was not inhibited, but root growth was reduced. Cd content in both bean root and shoot also decreased.

Keywords: cadmium pollution; phytotoxicity; oxalic acid; tomato; bean.

1 Introduction

Cadmium is one of environmental priority pollutants because of its toxicity, persistence and bioaccumulation in trace amounts. Since the 1970s there has been great interest in the behavior and the ultimate fate of Cd introduced into soil from anthropogenic sources. Not all forms of Cd has the same biological availability and toxicity in the soil. Cd activity is influenced by soil solution composition, soil E_H, soil pH, soil type and density of charge and organic matter content (John, 1972; Gadde, 1974; Gardiner, 1974; Forbes, 1976; Farrah, 1977; Street, 1977; Soon, 1981; Laxen, 1985). The water-soluble and ion-exchangeable forms readily come into soil solution. They are thought to be the most mobile and bioavailable forms to plants and for this reason Cd in the soil solution has been most extensively researched. Some studies have identified a significant Cd component associated with colloidal and particulate phases, possibly as a result of adsorption. This adsorption is an important process in minimizing Cd toxic potential (Laxen, 1983).

Previous studies have shown that plant growth is reduced or retarded by higher available Cd content. Kabata-Penbadias (Kabata, 1992) reported that the phytotoxicity of Cd, beyond interfering with normal metabolism of some micro nutrients, shows inhibitory effects on photosynthesis, disturbs transpiration and CO_2 fixation, and alters the permeability of cell membranes. Mckenney and Vrisacker (Mckenney, 1985) found that Cd is an effective and specific inhibitor of the biological reduction of NO_2^- to NO.

Hue *et al.* (Hue, 1986) examined whether the presence of organic acid in soil solution affected Al phytotoxicity, using short - term, split - root experiments with cotton taproots. The root growth in Al solutions containing no organic acid decreased exponentially, but elongated in Al solutions with the presence of organic acid. This indicates that some Al in the solution was complexed by short - chained aliphatic di - and tricarboxylic acids.

In general, there are wide variations in both concentrations and kinds of organic acids in soil solutions of different soils. Organic acids are products of microbial activity (Bruckert, 1970b), which is greater in forest litter than in cultivated soils. Bruckert (Bruckert, 1970a) reported that a water extract of an Ao horizon covered with pine litter contained 86.7 μmol/L oxalic, 13.5 μmol/L citric, 39.3 μmol/L malic, 9.9 μmol/L malonic, 23.3 μmol/L acetic acid, and approximately 60 μmol/L of succinic + lactic acid. Organic acids existing in soil solution may reduce Cd and other trace metals toxicity, but there is currently little supporting data.

The objectives of this study were to determine (i) the Cd toxicity to plant root and shoot growth, with varying nutrient solution Cd concentration, and (ii) the effects of presence of organic acid in solution on plant root and shoot growth, and Cd accumulation.

2 Materials and methods

2.1 Experimental plants

Greenhouse - grown tomato and green bean seedlings were used as experimental plants. Tomato seedlings of equal height were purchased from a supermarket. Root was washed to remove soil particles and then was cut to 6.0 cm. Green bean seeds were planted in polyethylene pots. After four weeks when plants were selected that had a height of 16 cm and root length of 4.5 cm, they were transplanted to culture solutions. The shoot height and root length of plants were measured after two weeks of growth in culture solution for tomato plants and three weeks for bean plants.

2.2 Nutrient solution and treatment

All plants were grown in Hoagland's # 1 nutrient solution (Hoagland and D. I. Arnon, 1950). Table 1 shows the compositions and pH of this solution. One liter nutrient solution was

Table 1 Nutrient solution 1

Solution, 1 mol/L	Nutrient solution, ml/L	Chemicals	Contents, mg/L
KH_2PO_4	1	$B(H_3BO_3)$	0.5
KNO_3	5	$Mn(MnCl_24H_3O)$	0.5
$Ca(NO_3)_2$	5	$Zn(ZnSO_47H_2O)$	0.05
$MgSO_4$	2	$Cu(CuSO_45H_2O)$	0.02
		$Mo(H_2MoO_4H_2O)$	0.01
		$Fe(FeSO_47H_2O)$	0.5

added to each polyethylene container. One end of tubing was connected with air tap and the another end put into the nutrient solution to supply fresh air during plants growth. Distilled water was added to keep culture solution near one liter because of some solution absorbed and transpired by plants.

A Cd standard solution of 1000 mg/L was made with $CdCl_2$ and used for adjusting Cd concentrations. Oxalic acid was used as a complexing agent (Bruckert, 1970a,b). The experimental design for tomato and green bean plants is given in Table 2.

Table 2 Treatment design for tomato and bean

Treatment	Tomato			Green bean		
	NS*	Cd, mg/L	OA**, μmol/L	NS*	Cd, mg/L	OA**, μmol/L
1	NS			NS		
2	NS	2.5		NS	2.0	
3	NS	5.0		NS	4.0	
4	NS	10		NS	8.0	
5	NS	2.5	50	NS	2.0	25
6	NS	5.0	50	NS	4.0	25
7	NS	10	50	NS	8.0	25
8	NS	2.5	100	NS	2.0	50
9	NS	5.0	100	NS	4.0	50
10	NS	10	100	NS	8.0	50

* NS: nutrient solution   ** OA: oxalic acid

Each treatment was replicated three times and the data were compared using ANOVA (SYSTAT, 1990). The regration analysis was done by PROC REG in SAS program.

After measuring root length and shoot height, the roots were washed with deionized water. Then the roots and shoots were separated, oven dried (70℃), weighed and ground. The ground root and shoot samples were digested by HNO_3-H_2O_2-HCl (EPA 3050 method) and Cd was determined by ICAP (Thermo Jarrel Ash ICAP 61E, Thermo Jarrel Ash, Franklin, MA).

3 Results and discussion

3.1 Growth reduction of plant and absorption by plant

3.1.1 Tomato

Elevated Cd concentrations in the nutrient solution from 0 to 10 mg/L, tomato root and shoot growth were reduced significantly. With Cd concentration increased, the dry weights of the various parts of tomato, the root length and shoot height are given in Tables 3 and 4. The results pointed that with Cd concentration increased, biomass decreased a 36%—59.4% compared to no Cd addition. The toxicity of Cd was higher on shoot (36.9%—62.7% reduction in weight) than on root (36.1%—51.7%). The root length decreased from 28 cm to 16 cm and the shoot height decreased from 23 cm to 15 cm. Roots were shorter and finer at 5 and 10 mg/L Cd. Roots were swollen and growth retarded. Plant became brown in 10 mg/L ppm of Cd level, and the tomato leaf was chlorotic with brown spots. At Cd concentration above 5 mg/L in nutrient solution, the tomato plants were still alive, but growth decreased dramatically.

Table 3 Effect of organic acid on Cd toxicity in tomato growth

Treatment	Roots		Shoots		Whole plant	
	Dry weitht, g	% reduction in weight	Dry weight, g	% reduction in weight	Dry weight, g	% reduction in weight.
1	1.36(0.23)	0.0	3.22(0.27)	0.0	4.58	0.0
2	0.87(0.11)a	36.1	2.03(0.22)	36.9	2.90	36.7
3	0.82(0.20)a	39.7	1.57(0.20)a	51.1	2.39	47.7
4	0.66(0.10)a	51.7	1.20(0.17)b	62.7	1.86	59.4
5	1.31(0.19)	3.9	2.45(0.28)	23.8	3.76	17.9
6	0.94(0.21)	30.6	1.98(0.13)a	38.5	2.92	36.1
7	0.80(0.08)a	41.2	1.33(0.14)b	58.6	2.03	52.0
8	1.27(0.27)	6.7	2.48(0.26)	22.8	3.75	18.1
9	0.88(0.13)a	35.5	1.95(0.20)	39.3	2.83	38.2
10	0.76(0.12)a	43.8	1.52(0.17)a	52.8	2.28	50.2

a: $P \leqslant 0.05$ b: $P \leqslant 0.01$

Table 4 The effect of organic acid on Cd toxicity in tomato root and shoot growth

Oxalic acid,	Cd, mg/L				Cd, mg/L			
μmol/L	0	2.5	5.0	10	0	2.5	5.0	10
	Shoot height (SD), cm				Root length (SD), cm			
0	23(1.3)i	16(1.7)a	16(0.6)a	15(2.2)a	28(1.6)	23(1.0)a	16.7(1.5)a	16(1.7)a
50		17(0.6)a	17(1.2)a	17(2.0)a		29(1.0)	23(1.6)	16(2.3)b
100		18.5(2.5)a	17(0)a	17(1.5)a		30(1.8)	18.7(0.6)b	16(1.7)b

a: $P \leqslant 0.05$ b: $P \leqslant 0.01$

Cd is non-essential to plant growth, but is effectively absorbed by both root and leaf systems (Kabata, 1992). This study found the same result (Fig. 1). There is an almost linear correlation between Cd content in root and shoot vs. Cd concentration in the nutrient solution. As Cd concentration increased from 0 to 10 mg/L, Cd in the tomato root increased from 4 to 3850 mg/kg, shoot accumulation increased from 1.5 to 280 mg/kg. This indicates that a great proportion of the Cd in the nutrient solution was accumulated by root tissues. About 15 times more Cd was accumulated by tomato root than by the shoot.

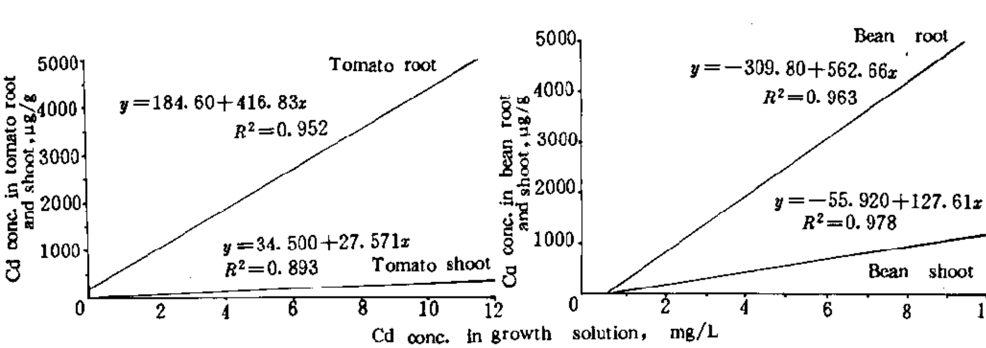

Fig. 1 The correlation between Cd in growth solution and roots and shoots of tomato and bean

3.1.2 Green bean

The green bean plant showed more sensitivity to Cd than the tomato plant. Also there was a linear correlation between Cd in root and shoot vs. Cd in the nutrient solution (Fig. 1). When Cd concentration increased from 2 to 8 mg/L in nutrient solution, there was a 35.5%—83.3% reduction in weight (Table 5). The root and shoot growth were reduced significantly, and the plants nearly died in 4 and 8 mg/L of Cd solution (Table 6). Cd absorption by bean roots was similar to the tomato plant, but bean shoot accumulated more Cd than tomato shoots. At Cd 8 mg/L, beans accumulated 1011 μg/g in shoots, however tomato shoots accumulated just 281 μg/g at 10 mg/L. This could explain why bean almost die at 4 and 8 mg/L Cd.

Table 5 Effect of organic acid on Cd toxicity in bean growth

Treatment	Roots		Shoots		Whole plant	
	Dry weitht, g	% reduction in weight	Dry weight, g	% reduction in weight	Dry weight, g	% reduction in weight
1	0.42(0.02)	0.0	1.15(0.08)	0.0	1.57	0.0
2	0.16(0.01)[a]	61.1	0.85(0.05)[a]	26.1	1.01	35.5
3	0.03(0.004)[b]	92.9	0.45(0.04)[b]	60.8	0.48	69.4
4	0.02(0.003)[b]	95.2	0.24(0.02)[b]	78.9	0.26	83.3
5	0.32(0.01)	23.8	1.16(0.09)	−0.9	1.48	5.7
6	0.26(0.008)	48.0	0.83(0.06)	27.9	1.09	30.8
7	0.02(0.002)[b]	95.2	0.25(0.03)[b]	78.3	0.27	82.8
8	0.30(0.03)	28.6	1.17(0.09)	−1.4	1.47	6.6
9	0.17(0.02)[a]	59.5	0.79(0.06)[a]	31.3	0.96	38.9
10	0.02(0.003)[b]	95.9	0.25(0.03)[b]	78.3	0.27	83.0

a: $P \leq 0.05$ b: $P \leq 0.01$

Table 6 The effect of organic acid on Cd toxicity in bean root and shoot growth

Oxalic acid, μmol/L	Cd, mg/L 0	2.0	4.0	8.0	Cd, mg/L 0	2.0	4.0	8.0
	Shoot height (SD), cm				Root length (SD), cm			
0	30(2.0)	26(0.6)[a]	15(1.0)[b]	12.3(1.5)[b]	23(1.9)	15.7(1.5)[a]	4.7(0.6)[b]	5.0(1.0)[b]
25		30(1.0)	28.5(1.2)	16(1.0)[b]		17.3(2.0)	15.3(3.5)	5.0(1.5)[b]
50		35(2.3)	25(3.0)[a]	18.3(2.1)[b]		19(1.6)	16.3(3.2)	5.0(0.2)[b]

a: $P \leq 0.05$ b: $P \leq 0.01$

3.2 The effect of organic acid in tomato and bean growth

When oxalic acid was present as complexer in Cd solutions, tomato and bean plants grew much better than with no addition of organic acid.

3.2.1 Tomato

In the 2.5 mg/L of Cd-nutrient solution, tomato root weight and length was 0.87 g and 23 cm, respectively. However, when 50 and 100 μmol/L oxalic acid was added, the weights of root and root length were not significantly different than in solution with no Cd (Tables 3 and 4). At the same Cd level, tomato shoot heights were 16 cm (Cd), 17 cm (Cd+50μmol/L oxalic acid) and 18.5 cm (Cd+100μmol/L oxalic acid, Table 4). No significant difference was found in shoot height between oxalic acid addition and no addition. However, the reduction of shoot weight was significantly decreased from 36. 9% to 23. 8% and 22. 8% when oxalic acid was added. This indicates that oxalic acid reduced Cd toxicity for root and shoot growth.

At 5 mg/L Cd in nutrient solution, the addition of 50 μmol/L oxalic acid resulted in root length increases from 16. 7 cm to 23 cm, shoot height were no different to the only Cd level, and the reduction of whole weight decreased from 47. 7% to 36. 1%. The addition of 100 μmol/L oxalic acid resulted in slightly increases in root length and shoot height from only Cd level. But the reduction of whole weight decreased from 47. 7% to 38. 1%. At 10 mg/L, the addition of 50 and 100 μmol/L oxalic acid did not reduce Cd root toxicity and slightly decreased Cd shoot toxicity. The presence of 50 and 100 μmol/L oxalic acid at less than 5 mg/L Cd in nutrient solution may have an effective detoxifying action for root and shoot growth. At higher Cd level, the detoxifying effect diminished or disappeared, and Cd toxic predominated. Using regression approach to two factor analysis of analysis variance in this study (Y: root dry weight, or shoot dry weight or whole plant dry weight, X_1: Cd concentration, and X_2: oxalic acid concentration).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_1^2 + b_5X_1^2 \quad .$$

The b_3 represents an interaction effect, it is −0. 000316, −0. 00002641 and −0. 000233 for dry weight of tomato root, shoot and whole plant, respectively. The negative value of b_3 pointed that addition of oxalic acid reduced Cd toxicity to tomato growth.

Fig. 2 show the relationship between Cd in growth solution and in tomato root and shoot. They indicate the addition of 50 and 100 μmol/L oxalic acid complexed some Cd and reduced the amount of Cd accumulated in the root at all Cd concentration below 10 mg/L. However, presence of 50 and 100 μmol/L oxalic acid did not reduce tomato shoot Cd accumulation. In fact, it is even higher in solutions containing oxalic acid. This may indicate that soluble Cd complexes, for example oxalic -Cd still can be absorbed by tomato roots, quickly transported to shoot and accumulated. However this larger molecule has a lower toxicity than uncomplexed Cd.

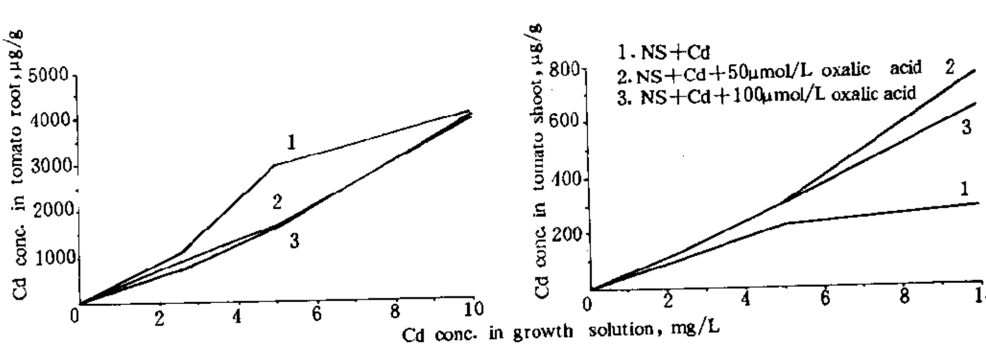

Fig. 2 The relationship between Cd in growth solution and roots and shoots of tomato and in the presence of oxalic acid

3. 2. 2 Green bean

The presence of 25 and 50 μmol/L oxalic acid in the Cd contaminated nutrient solution significantly detoxified solutions, and improved root and shoot growth in 2 and 4 mg/L Cd levels (Table 5 and 6). At 2 mg/L Cd, the reduction of whole weight, the root length and the shoot height were 35.5%, 15.7cm and 26 cm (only Cd), 5.7%, 17.3 cm and 30 cm (25 μmol/L oxalic acid), and 6.6%, 19 cm and 35 cm (50 μmol/L oxalic acid), respectively. At 4 ppm level, they were 69.4%, 4.7 cm and 15 cm (only Cd), 33.8%, 15.3 cm and 28.5 cm (25 μmol/L oxalic acid) and 51.8%, 16.3 cm and 25 cm (50 μmol/L oxalic acid), respectively. At 8 ppm of Cd, the addition of oxalic acid did not reduce the Cd toxicity. The root length and shoot height were almost the same as that of pretreatment, and the bean plants nearly died. The regression analysis for dry weight of bean root, shoot and whole plant using function of $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_2^2 + b_5X_1^2$ shows that b_3 is −0.000356, −0.001208 and −0.001564. These value indicated that Cd toxicity was retarded by adding oxalic acid.

The presence of 25 and 50 μmol/L oxalic acid inhibited Cd absorption by bean root and transport to shoot. The Cd content in root and shoot were lower in solutions with oxalic acid than without oxalic acid solutions (Fig. 3). Cd-complexes were not absorbed by bean root, thus Cd accumulation was reduced in plant tissues. Soluble species of Cd are always easily available to plants, but which Cd species can be absorbed depends on plant species.

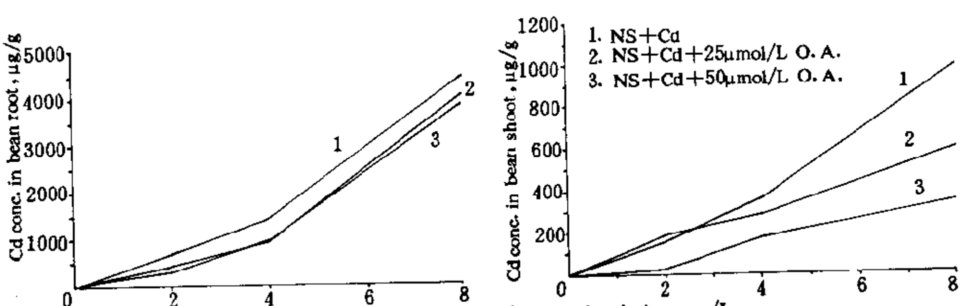

Fig. 3 The relationship between Cd in growth solution and roots and shoots of bean and in the presence of oxalic acid

4 Conclusions

Cd addition reduced or blocked root and shoot growth, thus decreasing the dry weight of plants. Higher Cd concentrations, result in higher plant toxicity. There was a nearly linear relationship between Cd in roots and shoots vs. Cd in the nutrient solution.

Roots accumulated more Cd than the shoots. Root and shoot Cd uptake varied greatly between plant species. Tomato roots accumulated more Cd than beans; bean shoots accumulated more Cd than tomatoes comparing both plant.

Organic acid in the nutrient solution can complex Cd, detoxifying the solution. However, Cd toxicity still occurs at high Cd concentrations. In contaminated soils, organic acid in the soil solution may reduce Cd toxicity.

Complexed Cd can be absorbed by root and transported to shoot though it appears to be less toxicity complexed Cd absorption depends on plant species.

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(Received January 17, 1994)