BioMetals 1992, **5**, 173–178

Interactive effects of cadmium, lead and zinc on root growth of two metal tolerant genotypes of *Holcus lanatus* L.

L. Symeonidis & S. Karataglis

Department of Botany, University of Thessaloniki, Thessaloniki, Greece

Received 20 December 1991; accepted for publication 15 May 1992

Lead and zinc tolerant genotypes of *Holcus lanatus* L. were grown in culture solution at different cadmium, lead and zinc concentrations, and combinations. In all treatments, an increased inhibition of root length with increasing concentrations of heavy metals was observed. Growth of genotype 1 was better than that of genotype 2 in all treatments, suggesting that genotype 1 is more tolerant. The better root growth of genotype 1, at different cadmium concentrations, than that of genotype 2, indicated the existence of a co-tolerance or greater tolerance of genotype 1 to cadmium. Heavy metal combinations resulted in increased lead or zinc uptake by plants, while cadmium was decreased. In a lead–zinc combination, decreased lead and increased zinc uptake were detected. The different interactive effects of heavy metals on root growth of genotype 1 (additive or synergistic) and genotype 2 (additive or antagonistic) may suggest their differential susceptibility to the above metals.

Keywords: cadmium, heavy metal interactions, *Holcus lanatus* L., lead, zinc

Introduction

Although some heavy metals, e.g. copper and zinc, are essential for plant growth, they are generally toxic and can ultimately cause the death of plants when present at elevated levels in soils (Antonovics *et al.* 1971, Smith & Bradshaw 1979).

In many cases, metalliferous environments are contaminated by more than one metal in potentially toxic concentrations (Ernst, 1974, Hutchinson 1975, Davies & White 1981, Karataglis 1982, Wallace 1982). However, plants growing in an environment polluted with heavy metals are often able to evolve metal tolerant ecotypes (Ernst 1990).

A number of authors documented responses of plants to combinations of metals in soils or growth solution. This multiple metal stress was found to be:

(i) *Additive*, i.e. relative growth under conditions of multiple metal stress is equal to the product of the relative growth produced by the individual metals in isolation (McGrath *et al.* 1980, Wallace *et al.* 1980, 1981, Allison & Dzialo 1981, Burton *et al.* 1986, Taylor 1989).

(ii) *Antagonistic*, i.e. relative growth under conditions of multiple metal stress is greater than that of the product of the relative growth produced by the individual metals in isolation (McGrath *et al.* 1980, Wallace *et al.* 1980, Wallace 1982, Wallace and Berry 1983, Taylor 1989, Taylor & Stadt 1990).

(iii) *Synergistic*, i.e. relative growth under conditions of multiple metal stress is less than that of the product of the relative growth produced by the individual metals in isolation (Wu & Antonovics 1975, Hassett *et al.* 1976, Carlson & Bazzaz 1977, Wallace *et al.* 1980, Wallace 1982, Wallace & Berry 1983).

The rooting test is normally used to assess the influence of heavy metals on plants, since Bradshaw (1952) and Wilkins (1957) demonstrated that root growth was particularly sensitive to metals. Therefore, the individual and combined impact of contaminating metals should be determined by measuring the individual and interactive effects of these metals on root growth.

This study aims to elucidate the influence of

Address for correspondence: L. Symeonidis, Department of Botany, University of Thessaloniki, 54006 Thessaloniki, Greece.

cadmium, lead and zinc, supplied separately and in combinations, on root growth of *Holcus lanatus* L. and on the uptake of metals.

Materials and methods

Plants of two genotypes of *H. lanatus* L. were selected from a lead–zinc mine area 'Mandem Lakkos' in Chalkidiki, Greece.

After several weeks growth in normal soil, tillers were separated and, after root excision, 15 tillers (three replicates of five tillers) in each treatment from each genotype were grown in a growth room at 22 ± 1 °C with a 16 h photoperiod, in a solution containing 0.5 $g l^{-1}$ $Ca(NO_3)_2 \cdot 4H_2O$ with or without the appropriate metal levels of cadmium, lead or zinc, and combinations of them (Table 1). The pH of the growth solution was 5.3 ± 0.3 and solutions were changed every 60 h to allow for aeration and to maintain the metal concentration. After 14 days the length of the longest root of each tiller at each metal concentration was measured.

Root length and relative root growth were used to express the degree of tolerance and patterns of heavy metal interactions. Plants were divided into roots and shoots, and carefully washed in distilled water. Plant and soil samples were dried at 80 °C. They were then treated with a nitric acid:perchloric acid (4:1) solution, and analyzed for cadmium, lead and zinc with an atomic absorption spectrophotometer.

Results

High amounts of lead and zinc were found in the rhizosphere soil of genotypes 1 and 2, while cadmium concentrations were low, but elevated, in comparison with those measured in normal soils (Table 2). In both genotypes a negative correlation between root length and the concentration of heavy metals in culture solution was observed (Figure 1 and Table 3).

Root length and relative root growth (percent of control) were higher in genotype 1 than in genotype 2 in all treatments (Figure 1 and Table 3). Differences in root length between genotypes (except for the controls) were significant [confidence limits (CL) 95%].

Table 2. Cadmium, lead and zinc concentrations (p.p.m.) in soil samples

Soil samples		Metals		
		cadmium	lead	zinc
(Genotype 1)	1	20	4600	5200
(Genotype 2)	2	16	3820	3500

In genotype 1, the interactive effects of both cadmium + lead and lead + zinc could be described as additive, since the relative root lengths were 59% and 47%, respectively, which were almost equal to the calculated values of 63% (90% × 70%) and 50% (70% × 71%) (Figure 1 and Table 3). Cadmium + zinc and cadmium + lead + zinc interaction effects were synergistic [i.e. the relative root lengths of 44% and 31% for cadmium + zinc and cadmium + lead + zinc, respectively, were less than that of the calculated values of 64% (90% × 71%) and 45% (90% × 70% × 71%)] (Figure 1 and Table 3).

Likewise, additive or nearly additive interaction effects were found in genotype 2 for cadmium + lead, cadmium + zinc and lead + zinc combinations. When cadmium, lead and zinc were supplied simultaneously, the interaction effect on root growth of genotype 2 was antagonistic [i.e the relative root length of 20% was greater than that of the calculated value of 14% (61% × 50% × 46%)] (Figure 1 and Table 3).

A positive relation was found between the heavy metal content of plants from genotype 1 and the concentration in the culture solution (Table 4). When cadmium was supplied in combination with lead or zinc alone the cadmium uptake by plants was inhibited, while there was a simultaneous increase in the uptake of zinc and, particularly, of lead (synergistic type effect). Transport of zinc or lead to shoots showed the same synergistic type effect (Table 4). When lead and zinc were simultaneously supplied, lead uptake was decreased (antagonistic type effect), while that of zinc was increased (synergistic type effect) in comparison with the uptake in the separately applied metals. Transport of lead or zinc to

Table 1. Supplied salts, and the concentrations of cadmium, lead, zinc and their combinations (p.p.m.) used in rooting tests

Salt used	Metal concentration				Combination			
Cadmium ($3CdSO_4 \cdot 8H_2O$)	0	1	3	6	1	1	–	1
Lead ($Pb(NO_3)_2$)	0	6	18	36	6	–	6	6
Zinc ($ZnSO_4 \cdot 7H_2O$)	0	4	12	24	–	4	4	4

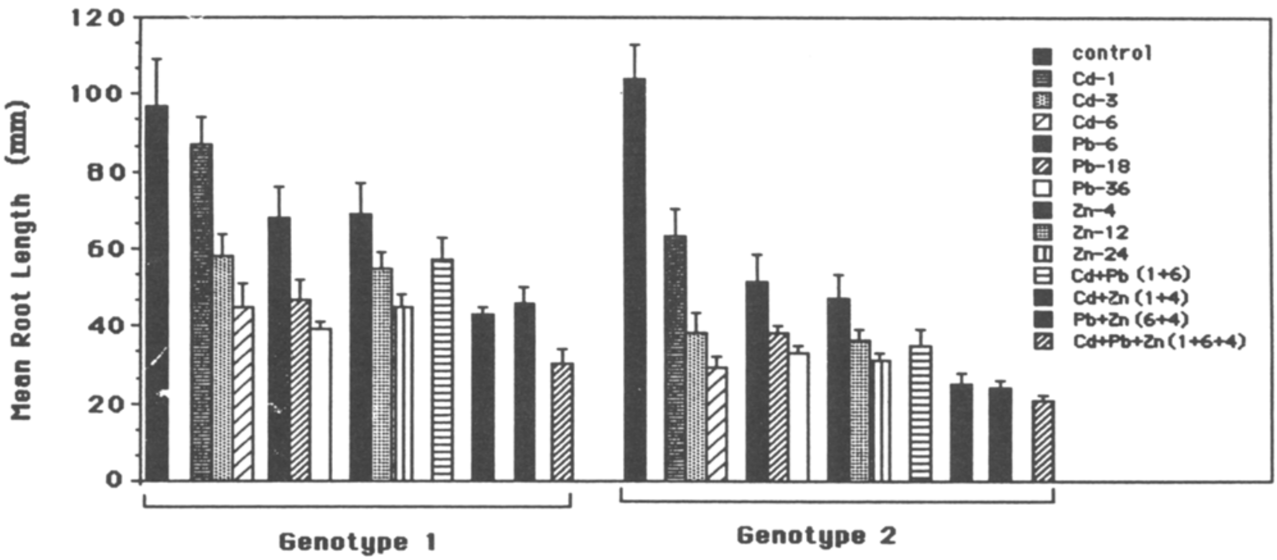

Figure 1. Mean root length (mm) of genotypes 1 and 2 at different cadmium, lead and zinc concentrations, in nutrient solution.

Table 3. Root length and relative root growth (%) of genotypes 1 and 2 grown in nutrient solution with different heavy metal concentrations (n = 15, results, except for controls, are significantly different, CL 95%)

Treatments	Root length (mm)		Relative root growth (%)		Calculated relative root growth (%)	
	genotype 1	genotype 2	genotype 1	genotype 2	genotype 1	genotype 2
Control	97 ± 11	103 ± 9	100	100	—	—
Cadmium (p.p.m.)						
1	87 ± 7	63 ± 7	90	61	—	—
3	58 ± 6	38 ± 5	60	37	—	—
6	45 ± 6	29 ± 3	46	28	—	—
Lead (p.p.m.)						
6	68 ± 8	51 ± 7	70	50	—	—
18	47 ± 5	38 ± 2	48	37	—	—
36	39 ± 2	33 ± 2	40	32	—	—
Zinc (p.p.m.)						
4	69 ± 8	47 ± 6	71	46	—	—
12	55 ± 4	36 ± 3	57	35	—	—
24	45 ± 3	31 ± 2	46	30	—	—
Cadmium + lead (1 + 6)	57 ± 6	35 ± 4	59	34	63	31
Cadmium + zinc (1 + 4)	43 ± 2	25 ± 3	44	24	64	28
Lead + zinc (6 + 4)	46 ± 4	24 ± 2	47	23	50	23
Cadmium + lead + zinc (1 + 6 + 4)	30 ± 4	21 ± 1	31	20	45	14

Table 4. Heavy metal contents in roots and shoots of genotype 1 in relation to heavy metal concentrations in culture solution

Treatments	Heavy metal content (p.p.m.)					
	root			shoot		
	cadmium	lead	zinc	cadmium	lead	zinc
Cadmium (p.p.m.)						
1	81	—[a]	—	11	—	—
3	96	—	—	14	—	—
6	153	—	—	22	—	—
Lead (p.p.m.)						
6	—	957	—	—	12	—
18	—	1538	—	—	37	—
36	—	2162	—	—	88	—
Zinc (p.p.m.)						
4	—	—	750	—	—	45
12	—	—	855	—	—	51
24	—	—	1132	—	—	76
Cadmium + lead (1 + 6)	4	1369	—	1	74	—
Cadmium + zinc (1 + 4)	3	—	951	1	—	59
Lead + zinc (6 + 4)	—	596	1017	—	55	71
Cadmium + lead + zinc (1 + 6 + 4)	4	1125	737	1	60	70

[a] Under detection limit.

shoots was increased compared with the transport when lead and zinc were individually supplied. Finally, when all three metals were simultaneously supplied, cadmium uptake was decreased, lead uptake was increased and zinc uptake was unaffected compared with the uptake of individually supplied metals. Transport of lead and zinc to shoots was increased while that of cadmium decreased (see Table 4).

Discussion

The present results confirm the occurrence of a differential susceptibility of the two genotypes to heavy metals. The greater root length and relative growth of genotype 1 in comparison with genotype 2 at all lead and zinc concentrations, single or in combination, may be the result of a better adaptation of genotype 1 to these elements, suggesting that genotype 1 is more tolerant than genotype 2. Karataglis (1982) found that there was a close relationship between the level of tolerance towards copper, zinc and lead within populations of *Agrostis tenuis*, and the contents of these metals in the soil.

The better root growth of genotype 1 upon cadmium supply (an element present in low, but elevated, concentrations in the soil) than that of genotype 2 must be due to a co-tolerance or greater tolerance of genotype 1 to cadmium. Co-tolerance has been mentioned in many other cases (Cox & Hutchinson 1981, Symeonidis *et al.* 1985, Verkleij & Bast-Cramer 1985, Verkleij *et al.* 1986).

Both lead and zinc interact with cadmium by depressing its uptake, while their uptake is increased (Table 4). Lead uptake is reduced when supplied together with zinc. There are reports of controversial interaction effects on uptake–transport processes of cadmium, lead and zinc for cadmium–lead, cadmium–zinc combinations as well as antagonistic ones for lead–zinc. The increased amount of nutrient elements in the shoot may be due to a competition of the above elements for the same sites, resulting in an increased translocation from roots to tops (see Kabata-Pendias & Pendias 1984). Similar heavy metal interactions have been reported in many other cases (Beckett & Davis 1978, Wallace 1982, Burton *et al.* 1986, Wallace & Abou-Zamzam 1989, Taylor 1989).

The two genotypes differed in their response to cadmium + zinc and cadmium + lead + zinc combination treatments. The differential responses of the two genotypes to the interaction of cadmium, lead and zinc may be due to the different sensitivity of these genotypes to the respective metals. Coughtrey & Martin (1978, 1979) also found differential responses on root extension and metal uptake of two populations of *H. lanatus* to cadmium, lead and zinc combinations. It seems possible that when growth reduction by one metal is great, the second metal has little additional or protective effect. This is how the observed antagonistic interactions of cadmium, lead and zinc in genotype 2 could be explained. The results of cadmium, lead and zinc combinations on root extension of *H. lanatus* reported by Coughtrey & Martin (1978) may be interpreted as synergistic (except for the lead–zinc combination) for the population tolerant to cadmium, lead and zinc, and as antagonistic for the non-tolerant population. Taylor & Stadt (1990) found that beyond a nickel concentration yielding a considerable root weight reduction of the secondary metal stress had little additional effect. Differential responses to cadmium + aluminum combination treatments were reported by McGrath *et al.* (1980) in two tolerant (cadmium-tolerant and aluminum-tolerant) races of *H. lanatus*. They found that the cadmium × aluminum interaction on root elongation was additive for the cadmium-tolerant race, but antagonistic for the aluminum-tolerant one. Wu & Antonovics (1975) and Davis & Carlton-Smith (1984) reported synergistic zinc × copper interactions in *Agrostis stolonifera* and *Lolium perenne*, respectively. Synergistic interaction of cadmium × cobalt × copper × manganese × nickel × zinc has been reported in *Phaseolus vulgaris* L. by Wallace (1982). Patterns of interactions between different phytotoxic metals have been reported as antagonistic in studies with *P. vulgaris* (Wallace 1982, Wallace & Berry 1983), *Glycine max* (Wallace *et al.* 1980), *H. lanatus* (McGrath *et al.* 1980) and *Zea mays* (Wallace *et al.* 1980). Antagonistic interations have been observed in *Triticum aestivum* by Taylor (1989) in a nickel × aluminum combination, by Taylor & Stadt (1990) in nickel × (cadmium, copper, manganese and zinc) combinations and by Miller *et al.* (1977) in a lead × cadmium interaction on leaf weight in *Z. mays*.

One must be careful, however, in interpreting primary growth data. Carlson & Bazzaz (1977) found that the form of interaction between lead and cadmium on growth of *Platanus occidentalis* varied with concentration. At low concentrations of lead and cadmium, interaction appeared additive while at higher concentrations it appeared synergistic.

Further study is needed in order to explain the differential patterns of cadmium, lead and zinc interactions on growth of *H. lanatus*, i.e. additive or synergistic in genotype 1 and additive or antagonistic in genotype 2.

References

Allison DW, Dzialo C. 1981 The influence of lead, cadmium, and nickel on the growth of rye grass and oats. *Plant Soil* **62**, 81–89.

Antonovics J, Bradshaw AD, Turner RG. 1971 Heavy metal tolerance in plants. *Adv Ecol Res* **7**, 1–85.

Beckett PHT, Davis RD. 1978 The additivity of the toxic effects of Cu, Ni and Zn in young barley. *New Phytol* **81**, 155–173.

Bradshaw AD. 1952 Populations of *Agrostis tenuis* resistant to lead and zinc poisoning. *Nature* **169**, 1098.

Burton KW, Morgan E, Roig A. 1986 Interactive effects of cadmium, copper and nickel on the growth of sitka spruce and studies of metal uptake from nutrient solution. *New Phytol* **103**, 549–557.

Carlson RW, Bazzaz FA. 1977 Growth reduction in American sycamore (*Platanus occidentalis* L.) caused by Pb–Cd interaction. *Environ Pollut* **12**, 243–253.

Coughtrey PJ, Martin MH. 1978 Tolerance of *Holcus lanatus* to lead, zinc and cadmium in factorial combination. *New Phytol* **81**, 147–154.

Coughtrey PJ, Martin MH. 1979 Cadmium, lead and zinc interactions and tolerance in two populations of *Holcus lanatus* L. grown in solution culture. *Environ Exp Botany* **19**, 285–290.

Cox R, Hutchinson TC. 1981 Multiple and co-tolerance in the grass *Deschampsia cespitosa*: adaptation, pre-adaptation and 'cost'. *J Plant Nutrit* **3**, 731–741.

Davies BE, White HM. 1981 Trace elements in vegetables grown on soils contaminated by base metal mining. *J Plant Nutrit* **3**, 387–396.

Davis RD, Carlton-Smith CH. 1984 An investigation into the phytotoxicity of zinc, copper and nickel using sewage sludge of controlled metal content. *Environ Pollut B* **8**, 163–185.

Ernst WHO. 1974 *Schwermetalvegetation der Erde*. Stuttgart: Fischer.

Ernst WHO. 1990 Mine vegetation in Europe. In: Shaw J, ed. *Heavy Metal Tolerance in Plants*. Boca Raton, FL: CRC Press; 21–37.

Hassett JJ, Miller JE, Koeppe DE. 1976 Interaction of lead and cadmium on maize root growth and uptake of lead and cadmium by roots. *Environ Pollut* **11**, 297–302.

Hutchinson TC. 1975 Heavy metal contamination of ecosystems caused by smelter activities in Canada. *Int Conf on Heavy Metals in the Environment*. Toronto, 28–31 October; C-316.

Kabata-Pendias A, Pendias H. 1984 *Trace Elements in Soils and Plants*. Boca Raton, FL: CRC Press.

Karataglis SS. 1982 Combined tolerance to copper, zinc and lead by populations of *Agrostis tenuis*. *Oikos* **38**, 234–241.

McGrath SP, Baker AJM, Morgan AN, Salmon WJ, Williams M. 1980 The effects of interactions between cadmium and aluminium on the growth of two metal-tolerant races of *Holcus lanatus* L. *Environ Pollut A* **23**, 267–277.

Miller JE, Hassett JJ, Koeppe DE. 1977 Interactions of lead and cadmium on metal uptake and growth of corn plants. *J Environ Qual* **6**, 18–20.

Smith RAH, Bradshaw AD. 1979 The use of metal tolerant plant populations for the reclamation of metalliferous wastes. *J Appl Ecol* **16**, 595–612.

Symeonidis L, McNeilly T, Bradshaw AD. 1985 Differential tolerance of three cultivars of *Agrostis capillaris* L. to cadmium, copper, lead, nickel and zinc. *New Phytol* **101**, 309–315.

Taylor GJ. 1989 Multiple metal stress in *Triticum aestivum*. Differentiation between additive, multiplicative, antagonistic, and synergistic effects. *Can J Bot* **67**, 2272–2276.

Taylor GJ, Stadt KJ. 1990 Interactive effects of cadmium, copper, manganese, nickel, and zinc on root growth of wheat (*Triticum aestivum*) in solution culture. In: Van Beusichem ML, ed. *11th Int Plant Nutrit Colloq.* Dordrecht: Kluwer; 317–322.

Verkleij JAC, Bast-Cramer WB. 1985 Co-tolerance and multiple heavy-metal tolerance in *Silene cucubalus* from different heavy-metal sites. In: *Proc 5th Int Conf Heavy Metals in the Environment*. Edinburgh: CEP Consultants; 174–176.

Verkleij JAC, Prast J, Ernst WHO. 1986 Different effects of cadmium on biomass-production and metal-uptake in cadmium-tolerant, co-tolerant and sensitive populations of *Silene cucubalus*. In: *Proc 2nd Int Conf Environmental Contamination*. Edinburgh: CEP Consultants; 27–29.

Wallace A. 1982 Additive, protective and synergistic effects on plants with excess trace elements. *Soil Sci* **133**, 319–323.

Wallace A, Abou-Zamzam AM. 1989 Low levels, but excesses, of five different trace elements singly and in combination, on interactions in bush beans grown in solution culture. *Soil Sci* **147**, 439–441.

Wallace A, Berry WL. 1983 Possible effects when two deficient essential elements are applied simultaneously. *J Plant Nutrit* **6**, 1013–1016.

Wallace A, Romney EM, Kinnear J, Alexander GV. 1980 Single and multiple trace metal excess effects on three different plan species. *J Plant Nutrit* **2**, 11–23.

Wallace A, Romney EM, Alexander GV. 1981 Multiple trace element toxicities in plants. *J Plant Nutrit* **3**, 257–263.

Wilkins DA. 1975 A technique for the measurement of lead tolerance in plants. *Nature* **180**, 37–38.

Wu L, Antonovics J. 1957 Zinc and copper uptake by *Agrostis stolonifera*, tolerant to both zinc and copper. *New Phytol* **75**, 231–237.