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Growth of Rye Grass and Fescue as Affected by Lead-Cadmium-Fertilizer Interaction[1]

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ABSTRACT

Rye grass (*Lolium perenne* L.) and red fescue (*Festuca rubra* L.) were grown from seed in fertilized (NPK, 12:6:6) and unfertilized Flanagan silt loam soil treated with Pb (0, 10, 100, 1,000, 5,000, 10,000 $\mu g\ g^{-1}$), Cd (0, 0.1, 1, 10, 50, 100 $\mu g\ g^{-1}$), or a combination of Pb plus Cd at a ratio of 100 Pb:1 Cd. Plant growth and heavy metal content of plants were measured at the end of three, consecutive, 10-day periods. Growth of Pb-treated plants did not begin to decrease until treatment concentrations reached 1,000 $\mu g\ g^{-1}$ Pb or above. Above a treatment concentration of 1,000 $\mu g\ g^{-1}$ Pb with or without added Cd, growth was reduced sharply in a log-linear fashion with increasing treatment concentrations. Some reduction in growth of plants treated with Cd alone occurred for fertilized fescue above 10 $\mu g\ g^{-1}$ Cd but not for rye until treatments above 50 $\mu g\ g^{-1}$. No reduction in growth was found for nonfertilized plants treated with Cd alone at the highest soil concentration (100 $\mu g\ g^{-1}$). Reduction in growth for plants treated with Pb + Cd was no lower than that of plants treated with Pb alone. While fertilization stimulated growth at treatment concentrations < 1,000 $\mu g\ g^{-1}$, it did not ameliorate the effects of Pb at higher concentrations. Fertilization reduced Pb content and uptake in rye ($P < 0.001$) but not in fescue. The Cd content of fertilized plants was greater ($P < 0.05$) than that of nonfertilized plants. Cadmium content of plants treated with Pb + Cd was greater ($P < 0.05$) than that of plants treated with Cd alone, while there was no difference in Pb content between Pb and Pb + Cd treatments.

Additional Index Words: Lolium perenne, Festuca rubra, synergism, antagonism, critical concentration.

Elevated concentrations of Pb and Cd have been found in both plants and soil near urban and rural roadways (Lagerwerff and Specht, 1970; Lagerwerff, 1971; Gish and Christensen, 1973; Solomon and Hartford, 1976; Hassett and Miller, 1977). Metal concentrations are greatest in the dust (up to 7,000 μg Pb/g) and surface soils (up to 15,000 μg Pb/g) near roadways and decrease with increasing distance from the road and increasing depth of soil. The ratio of Pb to Cd content in soils is also greatest near roadways (up to 945 $\mu g\ g^{-1}$ Pb:1 $\mu g\ g^{-1}$ Cd) and decreases to 50:1 at background metal concentrations [calculated from tabular values in Lagerwerff and Specht (1970) and Gish and Christensen (1973)]. In certain instances, metal contamination of roadside soils may be high enough to adversely affect plant growth (Jones et al., 1973).

Metal uptake and toxicity varies with soil characteristics. Soils with high cation exchange capacity and high phosphate levels tend to bind Pb and Cd, making them less available for uptake by plants and therefore less toxic (Miller et al., 1975a, 1976). Adding sulfur to a sulfur-poor soil decreases Pb uptake in rye grass (Jones et al., 1973). The interaction between metal uptake and soil fertility suggests that application of fertilizer may be a way to reduce metal toxicity of contaminated soil.

Environmental contaminants may act synergistically on plant growth. A synergistic response for Pb and Cd was found by Carlson and Bazzaz (1977) for root and shoot growth of American Sycamore (*Platanus occidentalis* L.) and by Miller et al. (1975b) and Hassett et al. (1976) for root growth of maize (*Zea mays* L.). In some instances the synergism may be due to increased metal uptake by plants when more than one toxic metal is in the soil (Miller et al., 1977).

Lawngrass species are the most common plants in urban environments. They are usually fertilized regularly and may have high Cd and Pb concentrations. Yet very little is known about their response to heavy metals. In this study we examine the effects of Pb and Cd both separately and together on the growth of two grass species, rye (*Lolium perenne* L.), and red fescue (*Festuca rubra* L.), at two different soil nutrient levels. Our objective was to test for interactions between Pb, Cd, and fertilization and metal uptake and plant growth.

MATERIALS AND METHODS

The experiment was factorially arranged with four factors and five replications. Factors included the two grass species, two fertilizer levels, three metal treatments, and six metal concentrations.

Soil was collected from the top 0.5 m of a Flanagan silt loam (a fine, montmorillonitic, mesic aquic Argindoll) far from major sources of Pb and Cd contamination. Soil pH was 5.9, cation exchange capacity 21 meq/100 g, total Pb content 6.1 $\mu g/g$, and total Cd content 0.4 $\mu g/g$. The soil was passed through a soil shredder-grinder and a 0.96-cm mesh screen and allowed to air dry. Plastic pots (16.5 cm diam) having large drainage holes were filled to 2.5-cm below the rim with 1,450 g of air-dry soil and placed in a glasshouse. Seed was sown at a density of 1.2 seeds/cm^2, and after germination was complete the plants were thinned to 1 plant/cm^2. Half the pots were fertilized with "Liquid Ortho Gro" (12:6:6) a the rate of 0.7 ml/pot (equivalent to 34 ml/m^2 of surface area) applied in 250 ml H_2O. Lead chloride and $CdCl_2$ in solution was then added to each pot to give five replications at each of six concentrations including 0, 10, 100, 1,000, 5,000, and 10,000 μg Pb/g air dry soil; 0, 0.1, 1.0, 10, 50, and 100 μg Cd/g; and combined treatment of 10 + 0.1, 100 + 1.0, 1,000 + 10, 5,000 + 50, and 10,000 + 100 for Pb + Cd, respectively. The metals were worked into the soil by repeated passing of leachate through the soil volume until there was no evidence of precipitate on the soil surface. After heavy metal addition and incorporation the plants were clipped to 2.5 cm in height on 28 July and the experiment begun. To simulate what might occur in a lawn the plants were clipped again at 10, 20, and 30 days after the first clipping to obtain harvests labelled 1, 2, and 3. The clippings were oven dried at 85°C to constant weight, a subsample dry ashed at 450°C for 5 hours, the ash dissolved in HCl, and Pb and Cd content determined by the Institute for Environmental Studies Analytical Laboratory at the University of Illinois using standard atomic absorption methods. Over the course of the experiment, daytime air temperature maxima varied from 25 to 35°C with nocturnal minima from 13 to 23°C. Relative humidity varied from 30 to 70% during the day and from 85 to 95% at night. Soil moisture was maintained at a high level throughout the experiment by daily application

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of deionized water to the soil surface until it just became visible in the saucer beneath each pot.

RESULTS AND DISCUSSION

Growth

In unfertilized soil at Pb treatments below 1,000 $\mu g\ g^{-1}$, growth of rye averaged 13.6, 12.6, and 10.8 g m^{-2}, and growth of fescue averaged 9.8, 8.9, and 14.5 g m^{-2} for harvests 1, 2, and 3, respectively. This is greater than the 6.9 g m^{-2} per 10-day period found for an irrigated bluegrass-fescue suburban lawn in southern California (Falk, 1976). Fertilizing with NPK in the present study caused an approximate doubling of growth at Pb treatment concentrations $<1{,}000\ \mu g\ g^{-1}$ for both species. Above a treatment concentration of 1,000 μg Pb g^{-1}, growth was reduced sharply in a log-linear fashion with increasing treatment concentration (Fig. 1). While soil Pb concentrations above 1,000 $\mu g\ g^{-1}$ presently occur only rarely in the environment, they were necessary in the present study to obtain reduced growth in rye and fescue. Treatment with Cd alone caused only a slight reduction in growth in treatment concentrations of 50 and 100 $\mu g\ g^{-1}$. In general the growth of plants treated with both Pb and Cd was not different from the growth of plants treated with Pb alone except for reduced growth of plants treated with $>1{,}000\ \mu g$ Pb g^{-1} + 10 μg Cd g^{-1}.

The results of an analysis of variance computed separately for each harvest revealed several significant differences and interactions. (i) Growth of rye was better than that of fescue in the first two harvests ($P < 0.05$), but not in the third harvest. (ii) The difference between growth at low and high metal concentrations was greater for fertilized plants than for nonfertilized plants (P

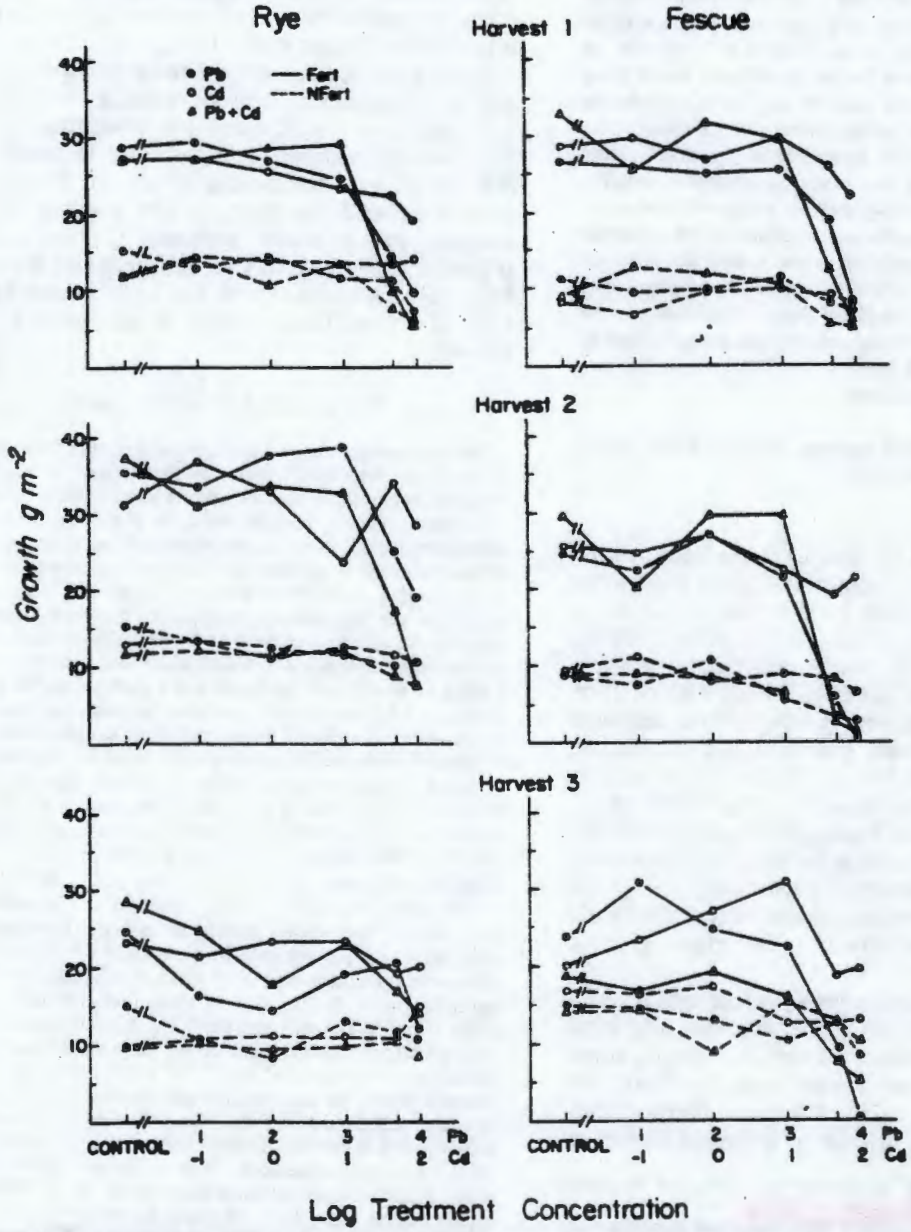

Fig. 1—Average growth of rye and fescue at different soil concentrations of Pb (●), Cd (○), and Pb + Cd (△), with and without added fertilizer.

< 0.005). This is caused by the fact that while fertilization stimulated growth at treatment concentrations $< 1{,}000$ μg Pb g^{-1}, the added fertilizer did not appreciably ameliorate the effects of Pb at higher treatment concentrations. (iii) Increased growth due to fertilization was greater for fescue than rye in harvests 1 and 2 but the reverse was true for harvest 3 ($P < 0.005$). Thus, it seems that fescue benefited more than rye near the beginning of the experiment from the higher fertility level, while the reverse was true for the third harvest. (iv) The interaction between species, fertilizer, and metal treatment was significant at $P < 0.05$. Growth of fertilized rye was higher for treatments with Pb and Cd alone than for treatment with Pb + Cd. For fertilized fescue, growth was higher for the Cd treatment than for the Pb alone and Pb + Cd treatments. For unfertilized plants, growth was approximately equal for all metal treatments.

These results suggest that (i) growth of rye in soil contaminated with either Pb or Cd alone may be increased with fertilization more readily than growth in soil containing both metals; (ii) growth of fescue in soil contaminated with Cd may be increased with fertilization more readily than growth in soil contaminated with either Pb alone or Pb + Cd; and (iii) differences between Pb- and Cd-contaminated soil will not be expressed by differential growth if soil fertility is low. Further experiments with a range of fertilizer concentrations are needed to verify these observations.

Metal Uptake

The Pb content of nonfertilized rye increased from ≤ 100 μg Pb g^{-1} for control plants to $> 9{,}000$ μg Pb g^{-1} at the highest treatment concentrations (Table 1). Similar results were found for fescue except that the maximum average concentrations were not as high. The Cd content of nonfertilized rye and fescue varied from < 1 μg Cd g^{-1} for control plants to > 200 μg Cd g^{-1} at the highest treatment concentration.

The effect of fertilization on Pb and Cd content was examined by expressing the metal content of fertilized plants as a fraction of the metal content of nonfertilized plants. This was done for each determination and the values averaged over all concentrations for each harvest (Table 2). Values less than unity indicate reduced metal content for fertilized plants with respect to nonfertilized plants and values greater than unity indicate increased metal content for fertilized plants. The significance of differences between variables was evaluated by a "t-test" statistic. The Pb content of fertilized rye was 0.44 to 0.69 of the value observed for nonfertilized plants. Reductions in Pb content have been found for other species in experiments where soil phosphorus levels have been increased (MacLean et al., 1969; Rolfe, 1973). For fescue the Pb content of fertilized plants varied from 0.76 to 1.89 of that for nonfertilized plants. For each species these values were not significantly different between harvests nor between plants treated with Pb alone and plants treated with Pb + Cd. Therefore, a mean was calculated for each species. The difference between rye (0.58) and fescue (1.38) was significant ($P < 0.001$), with the mean for rye also being significantly less than unity ($P < 0.005$). The difference between 1.12 (not significantly greater than unity) and 2.18 (greater than unity, $P < 0.001$) for total Pb uptake by rye and fescue, respectively, is also significant ($P < 0.001$). This suggests the presence of a basic difference between these species, with fertilization causing increased Pb content and uptake in fescue with respect to rye. The same kind of analysis was done for the effect of fertilization on Cd

Table 1—Metal content of clippings for each harvest and treatment under fertilized (F) and nonfertilized (NF) conditions. Each entry is an average for three replicate observations.

		Rye								Fescue							
						Pb + Cd								Pb + Cd			
		Pb		Cd		Pb		Cd		Pb		Cd		Pb		Cd	
Harvest	TC†	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF
		μg/g															
1	1	26	100	0.7	0.6	30	63	0.6	0.4	36	34	1.1	3.1	34	74	0.8	0.9
	2	74	140	3.0	1.5	130	230	3.7	4.2	56	50	0.3	1.4	29	76	2.0	1.6
	3	200	340	6.4	4.4	170	670	8.4	5.8	150	78	12.0	4.0	81	100	17.0	7.6
	4	1,140	2,220	50.0	26.0	530	1,880	29.0	19.0	160	400	31.0	2.7	190	430	60.0	39.0
	5	2,340	4,790	57.0	54.0	2,120	3,070	140.0	200.0	930	750	53.0	30.0	1,230	1,100	450.0	340.0
	6	4,710	11,300	105.0	100.0	3,490	9,400	210.0	220.0	4,640	3,520	100.0	36.0	8,780	1,950	660.0	560.0
2	1	58	41	No		52	88	No		39	17	0.5	0.3	30	21	0.4	1.1
	2	39	52	data		42	180	data		37	35	1.2	2.1	22	41	2.4	3.6
	3	40	130			55	110			48	46	9.2	4.6	34	68	10.0	7.5
	4	60	200			150	370			120	230	29.0	13.0	110	86	64.0	18.0
	5	250	880			270	630			600	790	30.0	45.0	950	250	510.0	46.0
	6	510	1,290			1,350	780			3,720	1,170	63.0	84.0	8,780	2,330	920.0	340.0
3	1	27	53	1.3	2.0	63	96	2.3	2.9	48	41	2.5	4.0	40	30	2.2	3.2
	2	65	97	3.9	7.3	70	120	4.3	3.8	62	74	1.9	2.5	86	100	1.8	1.8
	3	88	140	2.4	6.4	82	140	2.9	4.7	33	250	5.6	4.8	85	140	7.2	4.5
	4	96	170	15.0	8.8	88	600	16.0	20.0	100	200	13.0	6.6	130	190	27.0	25.0
	5	290	1,170	22.0	9.5	490	580	100.0	58.0	690	330	21.0	13.0	560	830	200.0	250.0
	6	5,450	3,580	48.0	22.0	810	980	280.0	250.0	3,800	710	36.0	22.0	2,530	6,310	280.0	240.0

† Treatment code (TC) 1–6 represents 0, 10, 100, 1,000, 5,000, and 10,000 μg Pb/g soil, respectively, for Pb-treated plants; 0, 0.1, 1, 10, 50, and 100 μg Cd/g soil, respectively, for Cd-treated plants; and 0 + 0, 10 + 0.1, 100 + 1, 1,000 + 10, 5,000 + 50, and 10,000 + 100 μg Pb + μg Cd/g soil, respectively, for plants treated with both Pb and Cd.

Table 2—Effect of fertilization on Pb content. Lead content of fertilized plants divided by Pb content of nonfertilized plants for each sample and averaged for each harvest.

	Rye		Fescue	
Harvest	Pb	Pb + Cd	Pb	Pb + Cd
1	0.47***	0.44***	1.18	1.29
2	0.58*	0.67	1.48	1.89
3	0.69	0.61*	1.68	0.76
Mean	0.55***		1.36	

*,*** Significantly different from unity at 5.0 and 0.1% levels, respectively.

Table 3—Effect of fertilization on Cd content. Cadmium content of fertilized plants divided by Cd content of nonfertilized plants for each sample and averaged for each harvest.

	Rye		Fescue	
Harvest	Pb	Pb + Cc	Cd	Pb + Cc
1	1.44*	1.17	3.23	1.40
2	N.D.†	N.D.	1.32	3.29
3	1.29	1.03	1.22	1.06
Mean	1.23*		1.93*	

* Significantly different from unity at 5% level.
† N.D. = no data.

content and uptake. The average ratio of Cd in fertilized to Cd in nonfertilized plants varied from 1.06 to 3.29 for the three harvests (Table 3). This ratio was not significantly different from unity except for harvest 1 of rye treated with Cd. The "t" statistic is sufficiently increased when the data from all harvests are combined to suggest that fertilization increased Cd content for both species. The total amount of Cd taken up by fertilized plants expressed as a fraction of the Cd taken up by nonfertilized plants and averaged over all observations was 3.27 for rye and 4.46 for fescue. Both values are significantly greater than unity of $P < 0.005$. Cadmium is more soluble in the presence of phosphate than Pb and therefore its availability for uptake should not be influenced by differences in soil fertility to the extent that Pb is (Miller et al., 1976). The greater Cd uptake and concentration for fertilized plants in the present study may have occurred simply as a passive consequence of the more favorable conditions for growth. A difference between species in fertilizer effect on Cd uptake and content similar to that observed for Pb was suggested by the data but excessive variability precluded the demonstration of statistical significance.

The concentration of Pb in plants treated with both Pb and Cd varied from 0.98 to 2.50 times that found in plants treated with Pb alone (Table 4). These values are not significantly different from unity, indicating that the presence of Cd in the soil at a concentration of 1/100th of that of Pb did not influence Pb content. The ratio of Cd in plants treated with both metals to the Cd content of plants treated with Cd alone, averaged for each harvest, varied from 1.41 to 2.82 for rye and 2.74 to 7.53 for fescue (Table 4). The values for each harvest were not significantly different from unity except for the first harvest of fertilized rye. However, the overall averages of 2.18 ($P < 0.05$) for rye and 5.11 ($P < 0.001$) for fescue are significantly greater than unity and suggest that Pb has caused an increase in Cd content above that of plants treated with Cd alone. The effect of Pb on Cd content has been noted previously for maize (Hassett et al., 1976, Miller et al., 1977). The difference between rye and fescue is also significant ($P < 0.01$) and is indicative of a basic difference between these species in their response to Cd in the presence of Pb. A similar analysis on Cd uptake produced nearly identical results, i.e., (i) the presence of Cd did not significantly influence Pb uptake in either rye or fescue; (ii) Cd uptake in plants treated with both Cd and Pb was greater than that measured for plants treated with Cd alone; and (iii) the ratio of Cd uptake by Pb + Cd-treated plants to uptake by plants treated with Cd alone was greater for fescue than for rye.

Table 4—Effect of Cd on Pb content and effect of Pb on Cd content. Metal content of plants treated with both Pb and Cd divided by metal content of plants treated with either Pb or Cd alone.

	(Pb in Pb + Cd)/Pb				(Cd in Pb + Cd)/Cb			
	Rye		Fescue		Rye		Fescue	
Harvest	F	NF	F	NF	F	NF	F	NF
1	0.96	1.09	1.07	1.35	1.41	1.90	4.31*	7.53
2	1.60	1.60	1.16	1.10	N.D.†	N.D.	6.28	2.24
3	1.19	1.39	1.26	2.50	2.59	2.82	4.25	6.07
Content mean	1.31		1.41		2.18*		5.11***	
Uptake mean	1.16		1.85		1.87*		3.47**	

*, **, *** Significantly different from unity at 5.0, 1.0, and 0.1% levels, respectively.
† N.D. = no data.

The sharp decline in growth that occurred for plants at Pb treatment concentrations above 1,000 $\mu g\ g^{-1}$, with or without Cd, (Fig. 1) was correlated with a sharp increase in Pb content (Table 1). The decrease in growth at high treatment concentrations appeared to be proportional to plant Pb content expressed as log $\mu g\ g^{-1}$, while plant growth at lower treatment levels appeared to be independent of plant Pb content. Beckett and Davis (1977) found this in their experiments with toxic metals and have suggested a method of data analysis that divides the growth curve into two parts. The first part is a horizontal line or plateau where growth is not reduced and the second a negatively sloping regression line where growth is inversely proportional to the log of leaf metal content. Each data set is objectively divided into a plateau region and a negatively sloping regression line by dividing the data set consecutively into two parts from below to above the point of apparent intersection and selecting the expression with the lowest variance. The intersection of these two curves is defined by Beckett and Davis as the "upper critical level" or leaf metal content at which growth begins to decline in response to a particular toxic metal. Critical levels determined for barley (Beckett and Davis, 1977) and other species (Davis and Beckett, 1978) were found to be quite similar at widely varying soil nutrient conditions. This prompted the authors to suggest that indicator plants with known critical levels grown in areas of suspected metal contamination could be used to determine the potential hazard of such areas. As the authors point out, this analysis depends upon the upper critical level being independent of all environmental parameters.

The results of the present study were readily adapted to the Beckett and Davis analysis. Critical levels of Pb

Table 5—Leaf metal content at which yield begins to decline (threshold concentration) for rye and fescue treated with Pb, Cd, or Pb + Cd.

Treatment	Metal	Harvest 1		Harvest 2		Harvest 3	
		F	NF	F	NF	F	NF
		μg/g					
			Rye				
Pb	Pb	730	950	84	61	560	2,000
Pb + Cd	Pb	570	1,800	91	400	72	600
Cd	Cd	29	≥117	ND†	ND	8	≥22
Cd + Pb	Cd	30	36	11	17	6	40
			Fescue				
Pb	Pb	70	410	50	100	46	300
Pb + Cd	Pb	130	300	96	25	51	420
Cd	Cd	38	≥37	18	≥91	19	≥22
Cd + Pb	Cd	59	26	64	4	19	177

† N.D. = no data.

and Cd and plateau values for growth are presented in Table 5 for rye and fescue. The differences between harvests and between fertilizer treatments are quite large for Pb-treated plants. The difference in critical concentrations of Cd are generally not as large but are still appreciable in certain instances. The wide variation observed for critical levels in this study does not appear to support the use of critical value analysis as a tool for the determination of potential metal toxicity of soil where large differences in fertility may exist.

Beckett and Davis (1978) extend their analysis to a consideration of how the threshold level of one toxic metal may be modified by the presence of a second metal. Yield is first plotted as a function of the tissue concentration of both metals. Then by noting the shape of the yield isopleths they distinguish between synergistic, additive, independent, and antagonistic effects of metal combinations. An examination of the data in Table 5 indicates that for threshold concentrations the interactions between Pb and Cd are either additive, independent, or antagonistic. Only for the second harvest of nonfertilized fescue is there an indication of synergism, i.e., at the upper critical level the Pb and Cd contents of Pb + Cd treated plants are both substantially lower than the respective metal contents of plants treated with each metal alone.

SUMMARY

Growth of rye and fescue were found to be relatively tolerant of Pb and Cd. Rye was more tolerant of Pb and Cd than fescue during the first 20 days of treatment, with no difference occurring thereafter. The decrease in growth at high treatment concentrations was proportional to plant metal content expressed as log $\mu g\ g^{-1}$, while plant growth at low treatment levels was independent of plant metal content. The growth of plants treated with both Pb and Cd was not different from the growth of plants treated with Pb alone. Fertilization stimulated growth at treatment concentrations < 1,000 μg Pb g^{-1}, but did not ameliorate the effects of Pb at higher concentrations. The increase in growth due to fertilization was greater for fescue than rye during the first 20 days of treatment, with the reverse being true for the 21- to 30-day period. Growth of rye in soil contaminated with either Pb or Cd alone was increased with fertilization more readily than soil contaminated with both metals. Growth of fescue in soil treated with Cd was increased with fertilization more readily than growth in soil treated with either Pb or Pb + Cd. Fertilization reduced Pb content in rye but not in fescue. Cadmium content was increased by fertilization for both species. Lead content was not increased by the presence of Cd. However, Cd content was increased by the presence of Pb in both species, with this effect being greater for fescue than for rye. The wide variation observed for critical levels (the concentration at which growth begins to be effected by Pb and Cd) does not support the use of critical value analysis as a tool for the determination of potential metal toxicity where large differences in soil fertility may exist.

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