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Heavy Metal Interaction for *Andropogon scoparius* and *Rudbeckia hirta* Grown on Soil from Urban and Rural Sites with Heavy Metals Additions[1]

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ABSTRACT

Little bluestem (*Andropogon scoparius*) and black-eyed Susan (*Rudbeckia hirta*) were grown in two soils with all combinations of Cd, Zn, Pb, and Cu at two levels each for 12 weeks. Germination and establishment were completely retarded by the addition of 2,000 μg/g Zn as $ZnCl_2$, which was due to a salt effect. Neither Cd nor Cu additions affected germination. A slight decrease in germination was noted for Pb additions of 900 μg/g which may also be associated with a salt effect. Cadmium at 10- and 20-μg/g addition rates did not affect top or root dry weight. Lead and Cu additions reduced shoot and root dry weight yields of *Andropogon scoparius*, root weights being more severely affected than shoot weights. Metal additions to the urban site soil did not reduce yields to the extent they did on the rural site soil. However, yields on the urban site soil control treatment were lower compared to those for the rural site control treatment.

DTPA extraction levels of heavy metals were not well correlated to plant concentrations for comparisons between the two soils. It was concluded that DTPA soil extraction may not be acceptable for metal availability comparisons among soils of differing pH.

Circumstantial evidence was found for both synergistic and antagonistic effects among the heavy metals. These were of a low level and no consistent response could be determined over species or soils.

Additional Index Words: cadmium, lead, copper, zinc.

In a previous experiment with Cd (Miles, 1978) the growth of several species grown in the greenhouse was stunted on a heavy metal-contaminated urban site soil. This was especially true for little bluestem (*Andropogon scoparius*) which was shown to have growth tolerance to Cd additions in an uncontaminated rural site soil. Total metal concentrations found in the urban site soil were 2.3, 442.5, 78.5, and 16.9 μg/g for Cd, Zn, Pb, and Cu, respectively. These are not unusually high values and perhaps could not individually account for the large reduction in plant dry weight noted for the urban site soil as compared to the rural site soil containing 0.3, 99.0, 12.6, and 5.6 μg/g for Cd, Zn, Pb, and Cu, respectively. This, however, needed to be empirically tested.

Several recent papers have investigated the possible interaction effects of heavy metals on each other. Dijkshoorn et al. (1975) found some evidence of a Zn by Cd interaction effect on growth. Carlson and Bazzaz (1977) found a positive Cd by Pb interaction (synergistic effect) on growth parameters for American sycamore (*Platanus occidentalis*). Hassett et al. (1976) also found a synergistic effect for Cd and Pb on maize root elongation and root dry weight. Miller et al. (1977) have also studied interactions of Pb and Cd and found significant interactions.

Other researchers have found interactions among metals which affect their availability or uptake (Findenegg and Broda, 1965; Lindsay, 1972; Brar and Sekhon, 1976; Chaudhry et al., 1973; Bingham et al., 1976; Haghiri, 1974).

Hassett et al. (1976) explained the synergistic Pb by Cd response they found as partially due to the elevated accumulation of metals in combination treatments. Such elevated metal accumulations may imply root damage (Turner, 1973). Competition between metals

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9274

for physiologically active sites have been speculated to be a possible factor in antagonistic metal interactions (Miller et al., 1977).

The research reported here was conducted to determine interaction effects of Cd, Zn, Pb, and Cu on plant growth.

METHODS

A factorial experiment was set up in the greenhouse utilizing two species, little bluestem (*Andropogon scoparius*) and black-eyed Susan (*Rudbeckia hirta*), and two soils with all combinations of Cd, Zn, Pb, and Cu at two levels each. This resulted in a 2^4 factorial experiment for each soil. Five blocks (replicate experiments) were set up using Plainfield sand (Mesic Typic Udipsamments) collected from a rural site with heavy metal addition levels of Cd at 0 and 20 μg/g, Zn at 0 and 4,000 μg/g, Pb at 0 and 900 μg/g, and Cu at 0 and 200 μg/g. Two blocks were set up using Oakville sand (Typic Udipsamments) collected from an urban site with heavy metal addition levels of Cd at 0 an 10 μg/g, Zn at 0 and 2,000 μg/g, Pb at 0 and 450 μg/g, and Cu at 0 and 100 μg/g. One pot for each of the species and each of the metals individually at an intermediate addition level (Cd at 10 μg/g, Zn at 2,000 μg/g, Pb at 450 μg/g, and Cu at 100 μg/g) was also set up within the first three rural site soil blocks to provide more information on the shape of the response curve for each metal. More detailed descriptions of the two soils were given in an earlier paper (Parker et al., 1978).

In a previous experiment (Miles and Parker, 1979) little bluestem was found to be the most tolerant to soil-added Cd of seven species tested and black-eyed Susan was the least tolerant. Therefore, these two species were used in this experiment to represent the range of heavy metal responses for species native to the urban site soil.

Metal concentrations given for the two soils in the introduction represent the mean concentration of the A horizon (top 14 cm). The top 2.5 cm of the urban site soil were several times greater than the mean of the A horizon (Parker et al., 1978). Since we were attempting to simulate the potential effects of the urban site soil on plant species, and since the top 2.5 cm of soil is believed critical for establishment and growth of plants, heavy metal addition levels were chosen to approximate the average concentrations (intermediate addition level) and the maximum concentrations (high addition level) found for these metals in the top 2.5 cm of soil on the urban site.

Except for Cu which was added as sulphate, all the metals were added as chlorides. To assess the potential effects of the high salt concentrations of the metal additions, several pots with $CaCl_2$ additions, of comparable Cl^- concentrations to those encountered in the heavy metal additions, were run concurrently with the experiment.

While past experience indicated that blocking over greenhouse bench position did not contribute to a reduction in experimental error, this experiment was begun in winter using supplemental artificial lighting to create a 16-hour photoperiod. Blocking was therefore utilized to remove variability due to variation in light intensity associated with greenhouse bench position.

A precipitate formed when the four metals were mixed in the solution and was particularly heavy for the Zn and Pb stock solutions. This necessitated addition of each metal be made separately to the soils in sequence. A 1-week incubation period was allowed to follow the addition of one metal before another was added so that any associated soil reactions would have time to equilibrate. After the final metal additions were made the soil was allowed to completely dry out. The soil was then removed from each pot, mechanically mixed, and repotted. After mixing and repotting in 11.4-cm standard plastic pots, the soil was again saturated using deionized water and allowed to incubate and partially dry before planting. Each pot containing 300 g of soil was placed in a 13.3-cm^2 plastic tray which was used to subirrigate, thereby minimizing wastout of seedlings and leaching loss of metals.

After seeding each pot with 40 seeds, the number of germinations were recorded every 2 to 3 days. When germination appeared to be complete the "0 day" was calculated. This was taken to be the day midway between the day the initial germination was recorded and the day the final germination was recorded. This was done separately for each pot and was used to represent the beginning of vegetative growth for each pot. Each pot was thinned to a maximum of five plants per pot 1 week after vegetative growth started. Two to 3 days later the pot was thinned to a maximum of three plants per pot and each plant labeled to distinguish it from the others in that pot. Beginning 7 days after growth initiation and weekly thereafter, until the experiment terminated, the number of individuals per pot was recorded. After 8 weeks the plants were separated into tops and roots, rinsed in deionized water, dried at 80°C, and weighed to the nearest 0.001 g.

Soils were analyzed for available heavy metals using DTPA extraction (W. L. Lindsay and W. A. Norvell. 1969. Development of a DTPA micronutrient soil test. Agron. Abstr. p. 84) with a 2:1 extractant to soil ratio and a 2-hour shaking time. Plant top and root samples were also analyzed for heavy metals using nitric acid digestion. Determinations were made using a Varian AA6 atomic absorption spectrophotometer. A hydrogen lamp background correction was used for Cd, Zn, and Pb. Extraction, digestion, and metal analysis runs were blocked on replicates in order to reduce the number of samples handled at one time.

RESULTS AND DISCUSSION

GERMINATION AND SURVIVAL

Germination for both species in both soils was negligible at 2,000 and 4,000 μg/g Zn addition levels. The few seeds which did germinate died before establishment. Similar germination occurred for the $CaCl_2$ addition pots. Thus the lack of germination for Zn treatment is concluded to be a salt effect resulting from Zn being added as $ZnCl_2$.

With the possible exception of Pb there was no consistently discernable effect of Cd, Pb, and Cu on germination. Since $ZnCl_2$ at 2,000 μg/g Zn completely retarded germination due to a possible salt effect, the germination effect noted for 900 μg/g Pb as $PbCl_2$ may be a salt effect.

Seedling survival was essentially complete over all treatment combinations except Zn at 2,000 and 4,000 μg/g on the urban site soil. This is consistent with the lack of mortality noted in a previous experiment for this soil (Miles, 1978). Mortality did occur for both species on the rural site soil which can be accounted for in part by treatment differences between the two soils. Intermediate levels for the single element heavy metal additions (i.e., Cd, 10; Pb, 450; Cu, 100 μg/g) on the rural site soil are comparable to the levels utilized on the urban site soil. Little bluestem on the rural site soil at these intermediate levels, like the urban site soil treatments, showed no mortality effect. Black-eyed Susan, however, did show mortality effects at the intermediate levels on the rural site soil. Thus, while part of the differences for survival can be accounted for by differences in metal levels between the two soils, there is some other soil factor, such as CEC or pH or another antagonism, which is counteracting the deleterious effects of heavy metals on survival for the urban site soil. Characteristics of the two soils are given in Table 1.

Black-eyed Susan mortality on the rural site soil was total with 900 μg/g Pb or 200 μg/g Cu addition. Mortality occurred within 3 weeks, indicating that the effect for black-eyed Susan is on seedling establishment. Cadmium (20 μg/g) did not affect survival of black-eyed Susan.

No mortality of little bluestem occurred with Cd at 20 μg/g in the rural site soil. Addition of Pb (900 μg/g) and Cu (200 μg/g) did decrease survival, but not totally. Copper had a greater effect on survival than did Pb. Changes in survival over time were due solely to Cu.

Table 1—Average values of several soil properties for the rural and urban site soils.

Soil property	Rural site soil	Urban site soil	n†
Total soil Cd, ppm	0.33	2.32	3
pH	4.80	7.82	4
P, kg/ha	56.6	24.4	4
K, kg/ha	88.0	16.3	4
N, $NH_4 + NO_3$, ppm	15.21	25.86	4
Organic matter, %	1.93	2.48	4
CEC, meq	9.28	12.22	4
Base saturation, %	24.28	97.36	4

† No. of samples.

RURAL SITE SOIL—PLANT WEIGHT EFFECTS

Cadmium additions to the rural site soil had no significant effect on black-eyed Susan top, root, or total dry weight (g per plant), or on root/shoot ratio, with overall averages of 0.40, 0.25, 0.65, and 0.63, respectively, for the 12-week growth period. A Pb addition of 450 ppm (the only Pb or Cu treatment surviving) did appear to reduce dry weight yield data by one-half to two-thirds, but since this result is based on only one surviving Pb treatment pot it can serve as no more than an indication of a potential effect.

Little bluestem on the rural site soil showed a significant Pb effect for all four weight variables analyzed but a significant Cd effect ($\alpha = 0.05$) only for top and total dry weight. Cadmium by Pb interaction (possible interactions with Cu being uninterpretable due to empty cells) was not significant for any plant weight variable. Effects of Cd and Pb thus appear to be additive for little bluestem on the rural site soil.

Linear regression equations were obtained (Table 2) for additions of each individual metal to the rural site soil for little bluestem. From an absolute standpoint (Table 2, 25% reduction concentration column) Cd is more effective in reducing yield than is a comparable Cu concentration. Lead is much less toxic than either Cd or Cu. Relative to the actual addition levels utilized in this experiment, however, Cu is more toxic than Pb, which is more toxic than Cd. Both Pb and Cu affect root weight more than top weight. Cadmium has no significant effect on the root/shoot ratio, however.

URBAN SITE SOIL—PLANT WEIGHT EFFECTS

Analysis of the urban site soil data would appear to offer a better test of metal interactions since there is no missing data, and thus all metal interactions can be analyzed. This is not the case, however, due to low replication and significant block effects. This is particularly true for black-eyed Susan because only for top dry weight are the block by metals interactions poolable to allow an estimate of experimental error with 4 degrees of freedom, which in itself is not adequate. While top weight ANOVA results do show some significant ($\alpha = 0.05$ level only) interactions, Student-Newman-Keuls tests on these interaction effects do not show any significance. It is therefore concluded from the results of this experiment that black-eyed Susan weight results are not significantly affected by metal additions to the urban site soil. Overall averages can be calculated for black-eyed Susan on the urban site soil of 0.54, 0.32, 0.86, and 0.59 for top, root, total dry weight per plant (g), and the root/shoot ratio, respectively.

Little bluestem on urban site soil showed no effect on root/shoot ratio from any added metal treatments (overall average, 0.51). The other variables (top, root, and total dry weight) all show significant Cd-Pb-Cu interactions (Table 3). Overall, Cu appears to have a negative effect on dry weight, which is not influenced by Cd and/or Pb. Cadmium and Pb both negatively influence growth singly but when combined have an antagonistic effect. For the urban site soil, effects of the three metals are not additive but antagonistic (Cd and Pb) or neutral (Cd and Pb with Cu).

Cu had the greatest negative effect on dry weight yield for little bluestem on the urban site soil. A comparable level of Cu in the rural site soil resulted in a dry weight yield (Table 3) similar to the urban site soil. These results warrant a closer look at Cu as a possible causative agent for the stunting effect noted for little bluestem in the urban site soil compared to the rural site soil for control treatments (Table 3) and in a previous experiment (Miles, 1978).

METAL AVAILABILITY

DTPA extraction shows a linear increase in metal availability with increasing additions of Cd, Pb, and Cu to the rural site soil (Table 4). It was assumed that species had no effect on metal availability, so extracted metals (Tables 4 and 5) represent averages over species and analysis blocks.

Zinc additions decreased the availability of Pb and Cu for both soils and slightly increased Cd availability. Since there was no germination and growth on the Zn treatments, there are no plant analyses to indicate

Table 2—Regression parameters and metal concentrations sufficient for a 25% reduction for *Andropogon scoparius* on a rural soil.

Metal	Weight variable, g	n	Intercept	Slope	R^2	25% reduction concentration, μg/g
Cd	Shoot	13	0.7279*	−0.0066	0.238	27.57
	Root	13	0.9163	−0.0123	0.262	18.62
	Total	13	1.6442	−0.0189	0.303	21.75
	Root/shoot ratio	13	-	NS	-	-
Pb	Shoot	13	0.8267	−0.0004	0.299	516.69
	Root	13	0.9455	−0.0008	0.743	595.47
	Total	13	1.7722	−0.0013	0.636	540.81
	Root/shoot ratio	13	1.2201	−0.0009	0.660	338.92
Cu	Shoot	9	0.7044	−0.0044	0.385	40.02
	Root	9	0.3451	−0.0056	0.756	37.73
	Total	9	1.5495	−0.0100	0.612	38.74
	Root/shoot ratio	9	1.2060	−0.0064	0.992	47.11

* All regression are significant at the $\alpha = 0.05$ level, except NS.

Table 3—Averages and (standard errors) for *Andropogon scoparius* shoot, root, and total dry weight for all treatment combi[illegible] on the urban site soil and at comparable metal levels on the rural site soil.

Soil	Treatment			Shoot		Root		Total	
	Cd	Pb	Cu	$\bar{y}$	$S\bar{y}$	$\bar{y}$	$S\bar{y}$	$\bar{y}$	[illegible]
	μg/g			g					
Urban†	-	-	-	0.377 a*	(0.018)	0.249 a	(0.092)	0.626 a	[illegible]
	10	-	-	0.214 b	(0.101)	0.132 b	(0.053)	0.346 c	[illegible]
	-	450	-	0.204 b	(0.062)	0.095 b	(0.018)	0.299 c	[illegible]
	10	450	-	0.324 a	(0.058)	0.135 b	(0.032)	0.459 b	[illegible]
	-	-	100	0.146 b	(0.098)	0.055 b	(0.025)	0.201 d	[illegible]
	10	-	100	0.130 b	(0.070)	0.070 b	(0.044)	0.201 d	[illegible]
	-	450	100	0.155 b	(0.062)	0.071 b	(0.026)	0.226 d	[illegible]
	10	150	100	0.160 b	(0.054)	0.076 b	(0.039)	0.237 d	[illegible]
Rural‡	-	-	-	0.741	(0.056)	0.902	(0.116)	1.644	[illegible]
	10	-	-	0.616	(0.072)	0.341	(0.118)	1.457	(0.179)
	-	450	-	0.926	(0.121)	0.708	(0.148)	1.634	[illegible]
	-	-	100	0.143	(0.056)	0.091	(0.043)	0.233	[illegible]

* Means followed by the same letter do not differ significantly at the $\alpha = 0.05$ level.
† $n = 2$.
‡ $n = 3$.

whether this is a real effect on availability or merely a Zn effect operating on the extractant.

A comparison of pH (Tables 4 and 5) reveals that there is about a three-unit difference between the two soils and that for both soils metal additions depress pH slightly. Comparable metal additions to each soil (10 μg/g Cd, 450 μg/g Pb, or 100 μg/g Cu) result in comparable availabilities for Cd, Pb, and Cu. This is in spite of the great difference between the two soils with respect to pH, which has been reported to affect availability (Lagerwerff, 1971; Fulkerson and Goeller, 1973; Linnman et al., 1973; Miller et al., 1975a, 1975b, 1976; DuPlessis and Burger, 1971; John, 1976; Street et al., 1977; Lindsay, 1972; Hemphill, 1972; Singh, 1974; F. F. Munshower. 1972. Cadmium compartmentation and cycling in a grassland ecosystem in the Deer Lodge Valley, Montana, Ph.D. Thesis, Univ. of Montana, Un[illegible] published.).

Similar metal availability for the two soils may [illegible] to buffering capacity of the extractant minimizing [illegible] ability differences between the two soils due to pH. [illegible] attribute of the DTPA extraction procedure would [illegible] to reduce its utility for comparisons of availability among soils of differing reaction. For such comparisons an extractant which could reflect availability differences due to the natural acidity of the soil would be [illegible] (Symeonides and McRae, 1977).

METALS IN PLANTS

The true test of metal availability, however, is [illegible] plant (Hemphill, 1972; Forescue and Marten, 1977). Plant analysis (Tables 6 and 7) indicates that com[illegible]

Table 4—DTPA extractable metals and pH for a rural site soil, averages and (standard errors).

Metals added						Metals extracted			
Zn	Cd	Pb	Cu	pH†	n	Cd	Zn	Pb	[illegible]
μg/g						μg/g			
-	-	-	-	4.90	6	0.10	9.18	3.84	[illegible]
						(0.01)	(1.84)	(0.27)	[illegible]
-	20	-	-	4.85	6	10.63	11.18	3.45	[illegible]
						(0.70)	(1.15)	(0.21)	[illegible]
-	-	900	-	4.40	5	0.13	11.38	520.40	[illegible]
						(0.01)	(2.51)	(27.81)	[illegible]
-	20	900	-	4.45	5	13.24	12.70	541.20	[illegible]
						(0.55)	(2.54)	(28.75)	[illegible]
-	-	-	200	4.60	4	0.08	6.32	2.32	[illegible]
						(0.01)	(1.47)	(0.21)	[illegible]
-	2	-	200	4.60	4	10.90	9.80	3.40	[illegible]
						(0.52)	(1.98)	(0.14)	[illegible]
-	-	900	200	4.30	4	0.14	12.85	506.50	[illegible]
						(0.01)	(0.87)	(25.31)	[illegible]
-	20	900	200	4.40	4	11.12	9.58	480.00	[illegible]
						(0.70)	(1.95)	(27.34)	[illegible]
-	10	-	-	-	6	5.30	10.02	3.78	[illegible]
						(0.27)	(1.84)	(0.15)	[illegible]
-	-	450	-	-	5	0.10	9.30	241.56	0.46
						(0.02)	(1.88)	(13.19)	[illegible]
-	-	-	100	-	5	0.09	8.06	3.18	[illegible]
						(0.02)	(1.51)	(0.31)	[illegible]
2,000	-	-	-	-	3	0.09	769.33	−0.15	[illegible]
						(0.03)	(77.71)	(0.36)	0.04
4,000	-	-	-	4.35	3	0.11	1,087.33	−0.18	0.09
						(0.06)	(107.92)	(0.08)	[illegible]

† $n = 2$ for all pH measurements. No $S\bar{y}$ given due to no variability in some cells.

Table 5—DTPA extractable metals and pH for an urban site soil: averages and (standard errors).

Metal added						Metals extracted			
Zn	Cd	Pb	Cu	pH†	n	Cd	Zn	Pb	Cu
μg/g						μg/g			
-	-	-	-	7.35	4	0.78	68.25	12.71	4.54
						(0.03)	(1.44)	(0.35)	(0.18)
-	10	-	-	7.75	4	5.75	70.00	13.10	4.32
						(0.32)	(3.24)	(0.72)	(0.06)
-	-	450	-	7.65	4	0.38	58.50	177.48	4.24
						(0.02)	(3.78)	(5.82)	(0.12)
-	10	450	-	7.70	4	4.32	72.50	186.98	4.87
						(0.41)	(3.52)	(7.07)	(0.18)
-	-	-	100	7.80	4	0.78	62.75	12.36	47.25
						(0.03)	(1.93)	(0.16)	(1.26)
-	10	-	100	7.70	4	5.40	59.25	12.34	48.10
						(0.29)	(3.30)	(0.67)	(1.06)
-	-	450	100	7.70	4	0.94	65.25	188.50	47.55
						(0.02)	(3.35)	(2.09)	(1.73)
-	10	450	100	7.65	5	6.14	70.00	196.12	48.20
						(0.33)	(3.00)	(11.59)	(1.87)
2,000	-	-	-	6.90‡	4	1.04	580.00	1.23	1.62
						(0.04)	(6.58)	(0.17)	(0.07)

† n = 2 for all pH measurements. No $S\bar{y}$ given due to no variability in some cells.
‡ n = 1.

able metal additions to the two soils, even though producing comparable DTPA extracted metals, did not produce comparable plant metal concentrations between the two soils. Plant metal concentrations were much lower on the urban site soil with comparable metal additions. This could well be a function of soil pH on metal availability to the plant. The Cd^{2+} and Zn^{2+} activity in soil can decrease 100-fold for each unit increase in pH (Lindsay, 1972; Street et al., 1977). The ability of a soil to sorb heavy metals is a major influence on plant accumulation of heavy metals and is predominately a function of CEC and pH (Miller et al., 1975a, 1975b, 1976; John et al., 1972). The added metals were more available than the native soil metals. The effects that can be associated with such metal additions therefore probably represent an overestimate of the effects that could be obtained with a comparable total innate concentration of that metal. This points out that care must

Table 6—Shoot and root average metal concentrations and (standard errors) for two samples, each of *Andropogon scoparius* and *Rudbeckia hirta* on an urban site soil.

Plant part	Metals added			Andropogon				Rudbeckia			
	Cd	Pb	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	Cu
				μg/g							
Shoot	-	-	-	0.85	102.75	9.48	6.72	7.10	212.80	10.76	6.40
				(0.11)	(12.35)	(0.70)	(0.42)	(0.30)	(24.00)	(2.29)	(0.34)
	10	-	-	2.48	116.75	12.96	7.29	45.34	223.50	8.62	8.94
				(1.13)	(36.85)	(7.40)	(1.56)	(3.16)	(15.50)	(0.58)	(1.56)
	-	450	-	0.92	111.75	96.58	8.07	12.73	262.05	51.14	9.50
				(0.06)	(15.85)	(60.28)	(0.21)	(0.97)	(29.95)	(3.92)	(0.34)
	10	450	-	3.08	111.40	27.71	7.54	58.36	305.50	95.22	11.23
				(0.42)	(7.40)	(2.29)	(0.36)	(5.14)	(131.50)	(51.18)	(4.59)
	-	-	100	0.46	120.05	10.10	17.62	8.00	230.80	22.29	16.20
				(0.24)	(12.95)	(1.30)	(3.72)	(0.08)	(21.05)	(12.27)	(0.48)
	10	-	100	2.47	192.00	21.80	16.19	54.62	301.15	12.18	9.65
				(0.04)	(87.30)	(14.81)	(4.64)	(19.72)	(36.85)	(3.24)	(1.24)
	-	450	100	1.13	145.85	28.16	16.32	17.24	325.05	53.71	18.82
				(0.32)	(6.75)	(6.26)	(2.42)	(6.46)	(124.05)	(8.66)	(5.74)
	10	450	100	3.08	123.20	24.88	14.28	67.40	209.50	47.70	11.97
				(0.03)	(4.70)	(2.56)	(1.62)	(6.30)	(45.50)	(2.30)	(0.89)
Root	-	-	-	10.22	804.00	76.04	50.59	7.43	836.50	181.35	36.05
				(0.44)	(35.00)	(20.70)	(13.41)	(1.77)	(77.50)	(37.45)	(17.15)
	10	-	-	50.45	487.55	39.77	47.63	37.55	961.25	148.25	72.71
				(2.35)	(21.55)	(41.55)	(16.79)	(9.35)	(189.25)	(11.75)	(1.79)
	-	450	-	16.59	657.35	330.21	43.48	9.19	1,010.50	452.05	37.56
				(5.20)	(52.35)	(43.79)	(10.89)	(0.68)	(241.50)	(318.35)	(5.62)
	10	450	-	99.70	702.50	349.92	51.38	37.85	575.00	761.85	56.02
				(2.70)	(30.50)	(3.38)	(0.36)	(4.95)	(53.00)	(32.25)	(7.62)
	-	-	100	14.00	548.80	43.44	209.50	8.69	1,021.00	182.60	388.60
				(2.22)	(25.20)	(17.12)	(49.80)	(0.98)	(106.00)	(23.20)	(2.90)
	10	-	100	86.02	666.00	67.24	235.25	34.10	620.00	125.32	287.55
				(31.38)	(114.00)	(8.16)	(34.15)	(10.90)	(327.00)	(44.48)	(84.85)
	-	450	100	22.36	820.55	589.98	131.92	14.35	810.10	889.15	352.06
				(0.36)	(27.45)	(33.02)	(5.32)	(6.93)	(212.10)	(162.55)	31.04)
	10	450	100	87.26	569.30	240.04	189.38	47.25	1,007.00	1,137.30	425.00
				(14.96)	(54.80)	(13.08)	(13.62)	(6.05)	(82.00)	(92.00)	(52.90)

Table 7—Shoot and root average metal concentrations and (standard errors) for *Andropogon scoparius* and *Rudbeckia* [illegible] on a rural site soil.

Plant part	Metals added			n	Andropogon				Rudbeckia			
	Cd	Pb	Cu		Cd	Zn	Pb	Cu	Cd	Zn	Pb	Cu
	μg/g				μg/g							
Shoot	-	-	-	3	0.47	165.63	10.06	3.32	7.55	366.67	37.15	[illegible]
					(0.07)	(39.76)	(0.99)	(0.88)	(1.03)	(245.56)	(22.91)	[illegible]
	20	-	-	3	30.50	189.33	10.38	5.02	593.73	1,010.67	23.99	[illegible]
					(1.25)	(18.32)	(1.29)	(0.23)	(77.45)	(293.06)	(13.44)	[illegible]
	-	300	100	2	1.32	94.25	462.10	3.30				
					(0.56)	5.95)	(142.80)	(0.63)				
	20	900	-	2	119.50	176.80	613.15	3.97				
					(3.00)	(13.80)	(27.85)	(0.38)				
	10	-	-	3	18.36	161.80	10.32	5.18	371.30	1,047.33	37.44	[illegible]
					(3.00)	(62.20)	(0.62)	(0.44)	(23.86)	(281.19)	(10.34)	[illegible]
	-	450	-	3†	0.99	145.47	259.60	1.91				
					(0.27)	(23.85)	(2.52)	(0.63)				
	-	-	100	2	0.78	169.30	15.18	30.66				
					(0.30)	(72.30)	(4.78)	(1.10)				
Root	-	-	-	3	5.64	315.30	58.32	23.06	6.73	558.67	89.87	79.42
					(1.59)	(96.01)	(20.48)	(2.39)	(1.78)	(302.27)	(28.88)	[illegible]
	20	-	-	3	236.43	314.33	50.13	22.24	332.33	399.67	94.36	[illegible]
					(31.72)	(47.02)	(15.80)	(2.80)	(61.74)	(115.30)	(12.82)	[illegible]
	-	900	-	2	9.46	344.15	318.54	21.33				
					(4.50)	(108.75)	(18.16)	(6.15)				
	20	900	-	2	792.15	896.35	365.10	17.12				
					(138.55)	(168.65)	(58.62)	(2.60)				
	10	-	-	3	128.70	246.96	79.78	19.42	174.13	463.67	79.84	[illegible]
					(22.00)	90.96)	(0.06)	(0.56)	(25.24)	(145.34)	(2.34)	[illegible]
	-	450	-	3†	7.31	299.07	867.30	12.82				
					(2.21)	(74.99)	(470.51)	(1.20)				
	-	-	100	2	3.82	295.65	16.58	266.55				
					(0.22)	53.35)	(0.22)	(25.65)				

† n = 2 for *Andropogon scoparius*.

be taken in choosing the form in which to add a metal. Dijkshoorn and Lampe (1975) found Zn and Cd about twice as available from sulphate forms than from sewage sludge. Results may be more meaningful if expressed on a soil available or a plant tissue concentration basis rather than on a total (or added) concentration basis which is not a necessarily reliable guide to the amount that is available to plants (Hemphill, 1972).

Metal additions increased the concentrations of the metal in both the tops and roots of both species on each soil (Table 6 and 7). Accumulation of metals was usually greater for black-eyed Susan than for little bluestem, irrespective of the soil. Accumulation of added metals at comparable addition rates was much greater for top and root concentrations on the rural site soil than on the urban site soil with the possible exception of root Cu. This helps to explain why survival was affected more for the rural site soil than for the urban site soil with comparable metal addition for black-eyed Susan. This difference in accumulation characteristics between the two soils can probably be accounted for by soil differences in pH, CEC, etc.

While Pettersson (1976) found vast differences in the mobility of metals within the plant for different species, most researchers have reported restricted transport from roots to top (Pettersson, 1976; Jarvis et al., 1976; Fulkerson and Goeller, 1973). This is not the case for black-eyed Susan where Cd is readily translocated from roots to shoots, with a root/shoot ratio <1. On the other hand little bluestem retains most of the cadmium in (or on) the roots, with a root/shoot ratio >1. While there is no noticeable difference in the Cd root/shoot ratio between the soils or addition levels for black-eyed Susan, Cd is more readily transported to the shoots of little bluestem on the rural site soil than on the urban site soil. This indicates that either differences in soil factors (pH, CEC) are influencing transport within the plant or are influencing root uptake or metal adsorption to the root. Lagerwerff (1971) hypothesized that the physiology of plant uptake and translocation of heavy metals might be a function of soil pH.

All the metals seem to have a more equitable distribution within the plant on the rural site soil than on the urban site soil. Copper is the only metal that shows a definite difference in the ratio between Cu-amended and unamended treatments, the amended Cu being more strongly associated with the root systems.

Copper content of the little bluestem root systems is comparable between the two soils with comparable additions (Tables 6 and 7). Both of these treatments, as stated previously, had similar dry weight yields (Table 3). A power curve ($y = ax^b$) can be fit through the urban site soil data for Cu additions relating top weight as a function of root Cu concentration ($a = 6.299$, $b = -0.6815$). This gives a good estimate of top weight for the root Cu concentrations of little bluestem on the urban site soil (0.43 and 0.16 g estimates for the unamended and Cu-amended urban site soil treatments, respectively, as compared to recorded measurements of 0.38 and 0.15 g).

CONCLUSIONS

With the possible exception of Cu (no data was obtained for Zn), neither soil nor plant metal concentrations adequately account for the stunting of growth

noted on the urban site soil. Copper can only be used to explain this weight reduction if a nonlinear response is assumed. Nonlinear response is a valid representation of heavy metal response (Miller et al., 1977), but considering the extent of the data presented here it is still only an assumption.

Some evidence of synergism and antagonism could be found. As these did not seem to be consistent but varied with species, soil, and plant variable analyzed we do not feel that the results provide conclusive proof that metal interaction effects occurred for the metal, soil, species combination used here. More work needs to be done to define the mechanisms of synergistic responses. It does not appear that the stunting noted for little bluestem on the urban site soil can be explained by either a linear combination of or a synergistic interaction among the metals investigated here. This cannot be adequately determined however until the possible effects of soil pH and CEC on metal uptake and transport can be quantified.

A synergism is defined as the total effect of an interaction exceeding the sum of the effects of each substance (Odum, 1971). The technique proposed by Carlson and Bazzaz (1977) to quantify synergistic interactions is not based on a summed effect, as synergism is defined, but on a multiplicative effect. This is not necessarily wrong. What is important is that the experimenter identify the criteria he uses to test for synergistic responses. Ideally there should be some conceptual basis for this criteria. With the recent increase in the number of papers presented on possible heavy metal interactions, perhaps concommitent work on the theoretical aspects of and the mechanisms of synergistic effects is also called for.

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