Cadmium and Zinc Uptake by Corn (*Zea mays* L.) with Repeated Applications of Sewage Sludge

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Annual applications of digested sewage sludge were made on plots of an acid silt loam soil and a calcareous strip mine spoil material for a period of 12 and 7 years, respectively, to determine their effects on concentrations of Cd and Zn in the leaves and grain of one particular corn hybrid. On the acid soil, leaves and grain of corn had Cd and Zn contents that were proportional to amounts of sludge-borne Cd and Zn applied during the previous growing season by furrow irrigation. But, repeated sludge applications on the same acid soil in successive years did not cause concentrations of Cd in leaves and grain of corn to increase further. Zinc concentrations were increased significantly only in leaves of plants growing on soil repeatedly irrigated with the two lower rates of sewage sludge. In contrast, repeated sludge applications on calcareous strip mine spoil resulted in additive increases of both Cd and Zn contents in corn leaves and grain. Since the uptake of heavy metals by growing plants generally decreases as soil pH is increased, these results were contrary to those expected. Concomitant applications of sludge-borne organic matter and phosphorus may have been the cause for enhanced uptake of Cd and Zn by corn in response to repeated sludge applications on calcareous strip mine spoil but not on the acid soil.

Task force members of the Council for Agricultural Science and Technology (CAST, 1980) recently reviewed published and unpublished data to assess the uptake of Cd and Zn by plants growing on soils treated with sewage sludges. They found the available data were inadequate to predict contents of these metals in plants when sludge-borne Cd and Zn were repeatedly added to soils.

Information concerning cumulative effects of Cd and Zn resulting from repeated sludge applications was obtained from two field studies where metalliferous digested sewage sludges from Chicago, IL, were applied annually on a silt loam soil for 12 years and a calcareous strip mine spoil for 7 years. These investigations were initiated to study yield responses and relationships between concentrations of inorganic chemical elements in plant tissues and amounts of sludge-borne elements applied each year. Concentrations of 19 elements in sludge, soil, and the leaves, grain, and stover of corn were determined each year, except for the first 2 years on Blount plots. The data for Cd and Zn were made available to the Council of Agricultural Science and Technology (CAST) task force members and a portion of these data was published in CAST Report No. 83 (CAST, 1980). However, these data were oversimplified, especially with regard to the pH differences that existed between the acidic soil and the strip-mined calcareous geological materials. The objective of this paper is to reexamine and provide a comprehensive interpretation of the Cd and Zn data collected from these two long-term studies.

MATERIALS AND METHODS

Anaerobically digested sewage sludge from the West-Southwest Wastewater Treatment Plant in Chicago, IL, was annually applied by furrow irrigation of corn, growing on lister ridges, in replicated (four) plots (6.1 × 12 m) of Blount silt loam soil (Aeric, ochraqualf, fine, illitic, mesic) and level strip mine spoil near Joliet and Canton, IL, respectively. The strip mine spoil had a $CaCO_3$ equivalent of 3.2%. At both sites liquid sludge applications of 0, 6.4, 12.7, and 25.4 mm, randomized within blocks, were made as soon and as often as weather conditions permitted. Each year the first application was made after plants reached a height of about 15 cm and applications were continued until about 3 weeks before harvest. Prior to spring tillage operations, Blount silt loam and strip mine spoil plots received broadcast applications of KCl to supply 134 and 112 kg of K/ha, respectively. Control plots were also fertilized with 268 kg of N/ha and 134 kg of P/ha on Blount silt loam and 336 kg of N/ha and 224 kg of P/ha on strip mine spoil. In Oct 1971, agricultural limestone was applied on Blount plots at rates calculated to raise the soil pH to 6.6. Rates varied from 1.7 mt/ha on some control plots to 10.1 mt/ha on some sludge-treated plots.

At the beginning and end of each irrigation event, samples of digested sludge were collected, dried at 110 °C for 24 h, and ground to pass an 80-mesh screen. Soil samples were collected each spring after the first tillage to a depth of 76 cm by using stainless steel tubes. Soil cores (six per plot) were segmented into 15.2-cm lengths, composited for a particular depth, dried (60 °C), crushed, split, and pulverized to pass a 60-mesh screen. Sludge samples (0.1 g) were heated to 500 °C for 24 h and dissolved in 0.1 N HCl for analysis by atomic absorption spectrophotometry to determine metal concentrations.

When about 10% of the plants had tasseled, leaves opposite and below the primary ear shoots were collected from the two center rows of each plot, washed in distilled water, dried at 60 °C, and ground in a Wiley mill to pass a 20-mesh screen. Subsamples of grain, hand-harvested from the two center rows to determine yields, were dried and ground in the same manner as leaf samples. Leaf and grain samples (2 g) were digested in concentrated HNO_3 at 90 °C, followed by $HClO_4$ at 200 °C, taken to dryness, dissolved in 0.1 N HNO_3, and analyzed for Cd and Zn concentrations by atomic absorption spectrophotometry with appropriate background correction.

Phosphorus concentrations in solution of digested materials were determined colorimetrically by using a vanadomolybdate finish (Olsen and Dean, 1965). Total organic carbon concentrations in soil and spoil were determined by the Walkley-Black procedure (Allison, 1965). The ammonium saturation method (Chapman, 1965) was used to determine the cation-exchange capacity (CEC) of the soil and strip mine spoil. Total nitrogen concentrations were determined by a semimicro-Kjeldahl method (Bremner, 1965). Determinations for pH were made with a combi-

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Table I. Amounts of Solids, Cd, Zn, and P Annually Applied as Constituents of Digested Sewage Sludge[a] Used To Irrigate Maximum-Treated Plots on Blount Silt Loam (B)[b] and Strip Mine Spoil (S)

	liquid sludge				accumulative[c]							
	total depth, mm		av solid contents, %		solids, mt/ha		P, mt/ha		Cd, kg/ha		Zn, kg/ha	
year	B	S	B	S	B	S	B	S	B	S	B	S
1968	171		3.0		51.5		1.3		11		317	
1969	254		1.9		99.8		3.2		28		787	
1970	229		2.3		152.5		5.2		48		1201	
1971	381		3.4		280.9		9.2		77		1806	
1972	127		2.0		306.5		10.0		81		1905	
1973	279	102	2.2	1.2	368.6	11.9	11.9	0.4	88	2	2122	60
1974	178	127	2.8	4.2	417.4	62.9	13.8	2.7	101	24	2358	315
1975	127	152	2.6	4.1	449.9	121.5	14.8	4.1	108	42	2499	568
1976	178	152	3.0	3.9	504.4	179.6	16.7	6.6	124	59	2716	846
1977	152	178	3.4	4.6	556.6	257.6	17.7	9.2	138	87	2969	1321
1978	178	152	3.9	4.4	625.1	321.9	19.5	11.4	157	111	3234	1655

[a] Sludge was applied by furrow irrigation at a maximum rate of 25.4 mm and appropriately lesser amounts on 0.25- and 0.50-maximum-treated plots as frequently as permitted by weather conditions. [b] Blount data through 1975 were previously published by Hinesly et al. (1977). [c] Accumulative rates include amounts applied during the specified year.

Table II. Corn Grain Yields (Adjusted to 15.5% Moisture) from Plots of Blount Silt Loam and Strip Mine Spoil with and without Annually Repeated Irrigations with Digested Sewage Sludge

	mt/ha for sludge irrigation rates									
	0 mm	6.4 mm	12.7 mm	25.4 mm		0 mm	6.4 mm	12.7 mm	25.4 mm	
year	Blount silt loam[a]				LSD[b]	strip mine spoil				LSD[b]
1968	4.16	6.03	7.16	7.02	n.s.[c]					
1969	8.96	9.34	9.42	9.44	n.s.					
1970	5.53	7.48	7.62	8.63	1.28					
1971	6.06	6.50	6.92	7.88	n.s.					
1972	8.94	8.62	8.99	8.82	n.s.					
1973	4.00	6.05	6.72	7.63	1.31	1.96	3.44	3.17	3.10	n.s.
1974	3.47	3.21	3.85	5.11	0.69	1.11	1.44	1.74	2.38	0.68
1975	8.15	9.36	9.44	9.43	n.s.	4.00	4.95	5.41	5.15	n.s.
1976	6.52	7.25	7.98	7.96	0.64	2.99	4.38	4.37	3.81	0.83
1977	5.28	4.84	5.57	6.33	n.s.	2.37	1.60	2.09	3.00	0.95
1978	5.73	6.24	6.54	7.32	0.56	4.00	2.82	3.14	3.03	0.83
1979	8.51	8.93	9.75	9.22	0.82					

[a] Data through 1975 were previously reported by Hinesly et al. (1977). [b] Least significant difference at $P \leqslant 0.05$. [c] n.s., not significant.

nation pH electrode from a slurry of equal proportions of soil and distilled water.

Relationships between concentrations of Cd and Zn in corn tissues and amounts of sludge-borne Cd and Zn applied on Blount and strip mine spoil were determined by regression analysis as described by Sokal and Rohlf (1969). More specifically, we used their outlined methods for "Computing regression with more than one value of y per value of x".

RESULTS

Amounts of liquid-digested sludge applied each of 11 years on Blount silt loam and six years on strip mine spoil through 1978 are shown in Table I for plots treated with the maximum rate of 25.4 mm/irrigation. Cumulative dry weight equivalents of total solids, P, Cd, and Zn, applied on maximum sludge-treated plots are also exhibited in Table I. Amounts of sludge solids and Zn applied on strip mine spoil during 6 years were about 50% of those applied on Blount soil during a period of 11 years. Amounts of sludge-borne P and Cd on strip mine spoil were 60 and 70%, respectively, of those applied on Blount plots. Sludge applied on strip mine spoil contained higher concentrations of P and Cd than that applied on Blount, even though the sludge came from the same source.

Corn grain yields were frequently higher on Blount plots treated with maximum and 0.50-maximum rates of sludge than on the highly fertilized control plots (Table II). On strip mine spoil, yields from maximum sludge treated plots were also sometimes higher than those from control plots and those that received 0.25-maximum applications. However, all yields were low compared to those from undisturbed soil and sludge applications did not markedly improve the productivity of the strip mine spoil. Two-way analysis of variance comparing treatments vs. years showed that the differences in grain yield between years were significant ($P \leq 0.01$) for both sets of plots. Stands were reduced to about 12000 plants/ha on strip mine spoil during the last year of the study, due to severe crusting prior to seedling emergence and extended drought after emergence. Because of the extremely low stands, yields were not determined.

Concentrations of Cd and Zn in corn leaves and grain from Blount plots were linearly regressed against amounts of these metals applied at each level of sludge treatment 2 years prior to, 1 year prior to, or in the year of crop harvest. The best fit (highest r^2) was between metal concentrations in plant tissues and amounts applied during the preceding year. This appeared to be logical since sludge-borne metals applied during a particular growing season were not incorporated into the soil until the next spring. For this reason only the sludge-borne Cd or Zn inputs during the previous growing season were considered when the data were analyzed to determine effects of annual loading rates on concentrations of these metals in corn tissues. At first, regression analyses were done separately

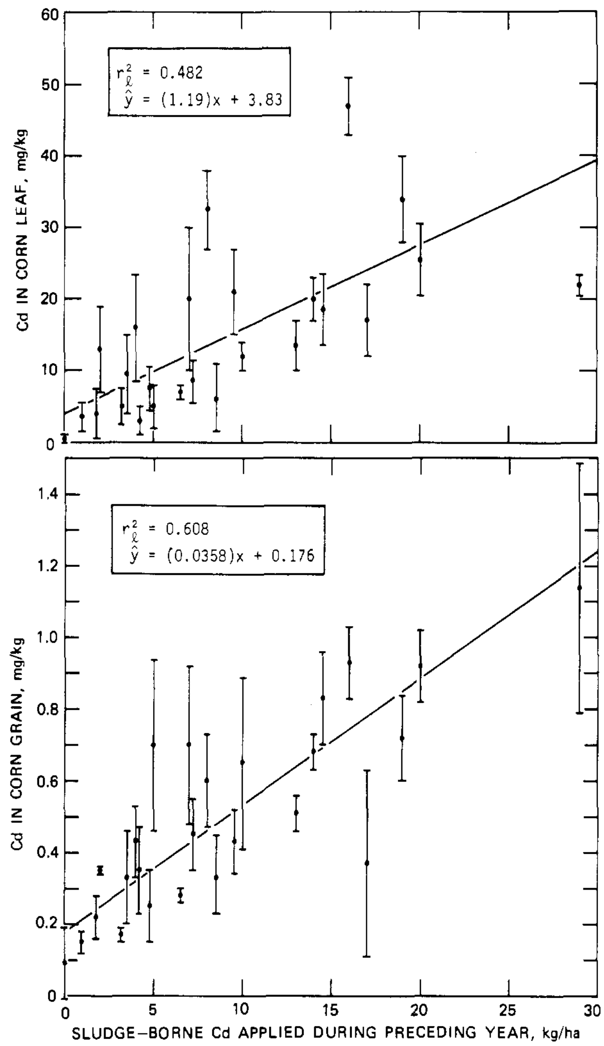

Figure 1. Relationship between amounts of Cd, supplied during the previous growing season as a constituent of sewage sludge, and concentrations (dry weight) of Cd in corn leaves and grain produced on Blount silt loam plots.

for 0.25-maximum-, 0.50-maximum-, and maximum-treated plots. However, since all three regressions were significant ($P \leq 0.05$), but not significantly different from each other, they were combined (along with data from check plots) into one overall regression.

In a similar regression analysis, the effects of cumulative loading rates on Cd and Zn concentrations in corn were determined by considering the sum of a particular sludge-borne metal applied during the initial year through the year just preceding the one in which plant samples were collected. However, regressions for 0.25-maximum-, 0.50-maximum-, and maximum-treated plots were not combined and are presented separately. This was necessary so that increased metal uptake due to annual sludge applications could be separated from those resulting from the accumulation of metals in the soil.

Cadmium and Zn data by year for corn leaf and grain are presented in Tables III and IV. Metal concentrations in corn tissues from Blount plots were not determined during the first 2 years of the study. Concentrations of Cd and Zn in leaves and grain of corn as a function of annual applications of the sludge-borne metals on the Blount soil are shown in Figures 1 and 2, respectively, along with the appropriate coefficients of determination (r^2) and linear regression equations. A significant ($P \leq 0.01$) proportion of the variation in concentrations of Cd and Zn was directly attributable to annual loading rates

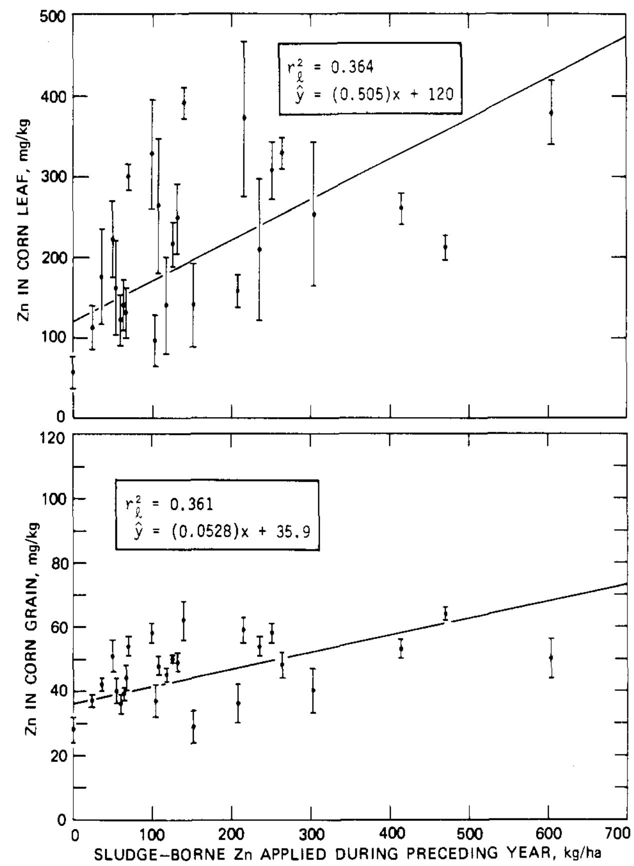

Figure 2. Relationship between amount of Zn, supplied during the previous growing season as a constituent of sewage sludge, and concentrations (dry weight) of Zn in corn leaves and grain produced on Blount silt loam plots.

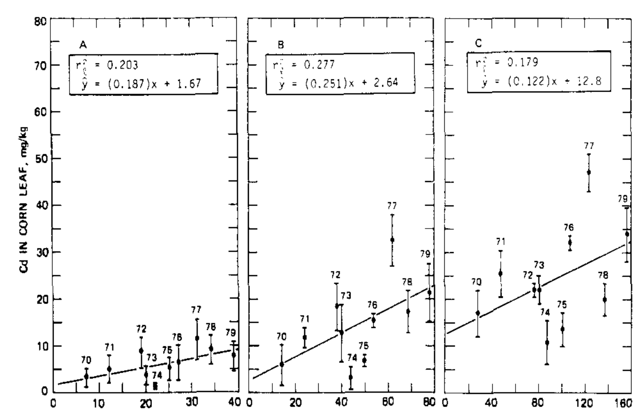

Figure 3. Concentrations (dry weight) of Cd in corn leaves from Blount silt loam plots treated with 0.25-maximum (A), 0.50-maximum (B), and maximum (C) loading rates of sewage sludge relative to accumulative amounts of Cd applied as a constituent of sludge. Sludge was applied during each of 12 successive growing seasons beginning in 1968. Analyses of plant tissue began in 1970.

of sludge-borne metals. Treatment sums of squares were partitioned into portions attributable to linear regression and unexplained deviations as outlined by Sokal and Rohlf (1969).

Cadmium concentrations in leaves and grain relative to the cumulative input of sludge-borne Cd on the Blount soil are presented in Figures 3 and 4, respectively. The least-squares linear regression line through data points were included to show trends even though it was found that neither leaf-Cd nor grain-Cd concentrations were significant functions of cumulative amounts of applied sludge-borne Cd. While the least-squares line showed a

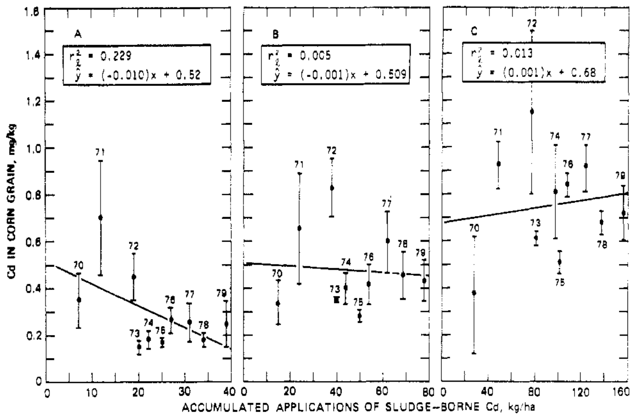

Figure 4. Concentrations (dry weight) of Cd in corn grain from Blount silt loam plots treated with 0.25-maximum (A), 0.50-maximum (B), and maximum (C) loading rates of sewage sludge relative to accumulative amounts of Cd applied as a constituent of sludge. Sludge was applied during each of 12 successive growing seasons beginning in 1968. Analyses of plant tissues began in 1970.

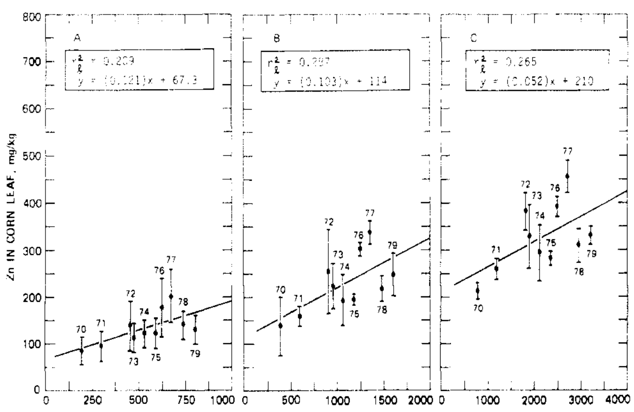

Figure 5. Concentrations (dry weight) of Zn in corn leaves from Blount silt loam plots treated with 0.25-maximum (A), 0.50-maximum (B), and maximum (C) loading rates of sewage sludge relative to accumulative amounts of Zn applied as a constituent of sludge. Sludge was applied during each of 12 successive growing seasons beginning in 1968. Analyses of plant tissues began in 1970.

trend for leaf-Cd concentration to increase with repeated annual applications of sludge-borne Cd, there was no indication that grain-Cd concentrations were affected beyond those that had already been established by applications made during the first 2 years of the study.

Zinc concentrations in leaves and grain, as affected by cumulative applications of the metal, are shown in Figures 5 and 6, r spectively. At the 0.25- (Figure 5A) and 0.50-maximum (Figure 5B) loading rates, Zn concentrations in leaves were significantly ($P \leq 0.05$) increased by cumulative applications of Zn, but the trend for a similar effect on maximum-treated plots was not statistically significant. Grain-Zn concentrations were unaffected by cumulative applications of sludge-borne Zn on Blount, regardless of the sludge loading rate.

Cadmium and Zn concentrations in corn leaf and grain grown on strip-mine spoil are also summarized in Tables III and IV. Cumulative applications of sludge-borne Cd on calcareous strip mine spoil (Figures 7 and 8) significantly ($P \leq 0.01$) increased concentrations of Cd in leaves and grain of the same corn hybrid (also Pioneer 3517) at all loading rates. Deviations from linear regression were significant ($P \leq 0.01$) only for Cd concentrations in leaves from the maximum-treated (Figure 7C) and in grain from the 0.50-maximum-treated plots (Figure 8B). As can be seen in Figures 9 and 10, concentrations of Zn in corn plant

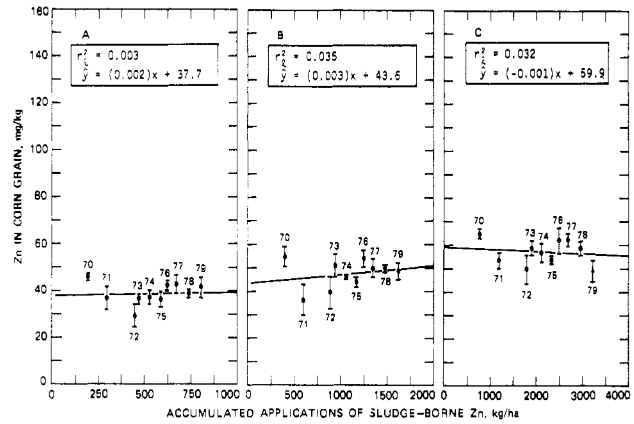

Figure 6. Concentrations (dry weight) of Zn in corn grain from Blount silt loam plots treated with 0.25-maximum (A), 0.50-maximum (B), and maximum (C) loading rates of sewage sludge relative to accumulative amounts of Zn applied as a constituent of sludge. Sludge was applied during each of 12 successive growing seasons beginning in 1968. Analyses of plant tissues began in 1970.

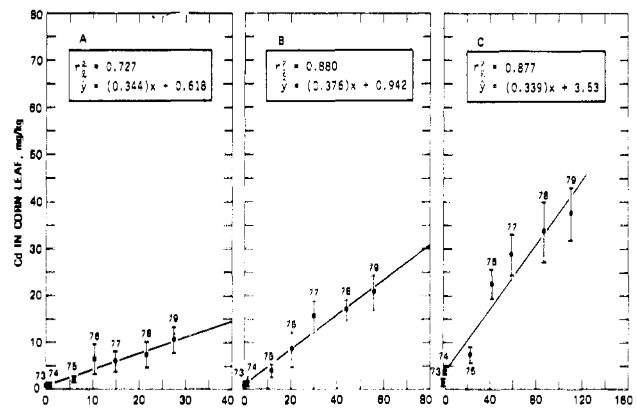

Figure 7. Concentrations (dry weight) of Cd in corn leaves from plots on strip mine spoil treated with 0.25-maximum (A), 0.50-maximum (B), and maximum (C) loading rates of sewage sludge relative to accumulative amounts of Cd applied as a constituent of sludge during each of seven successive growing seasons.

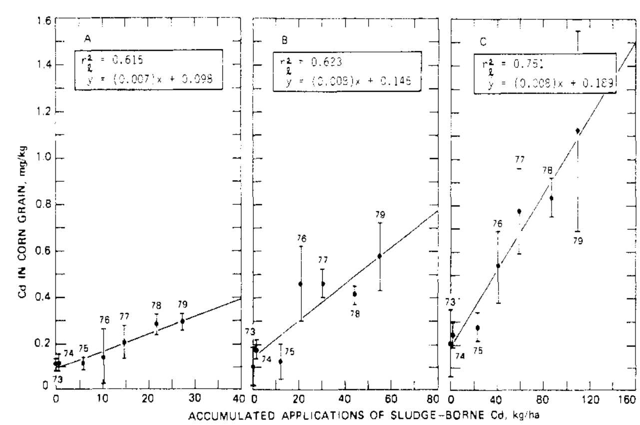

Figure 8. Concentrations (dry weight) of Cd in corn grain from plots on strip mine spoil treated with 0.25-maximum (A), 0.50-maximum (B), and maximum (C) loading rates of sewage sludge relative to accumulative amounts of Cd applied as a constituent of sludge during each of seven successive growing seasons.

tissues were not as dramatically affected by cumulative applications of sludge as were those for Cd. Concentrations of Zn in corn leaves and grain were less in 1979 than in 1978 samples. Because of the low survival of plants in 1979, tissue samples were collected from whole 8-row plots in contrast to previous years when samples were taken

Table III. Cadmium and Zinc Concentrations in Corn Leaf Grown on Blount Silt Loam and Strip Mine Spoil, with and without Repeated Applications of Sewage Sludge

		mg/kg (dry wt) for sludge irrigation rates									
		0 mm	6.4 mm	12.7 mm	25.4 mm		0 mm	6.4 mm	12.7 mm	25.4 mm	
analyte	year	Blount silt loam				LSD	strip mine spoil				LSD
Cd	1970	0.6	3.1	6.0	17.1	7.9[b]					
	1971	0.5	4.9	11.8	25.4	7.3[b]					
	1972	0.7	8.6	18.5	21.9	7.4[b]					
	1973	0.8	3.5	12.0	22.1	5.7[b]	0.34	0.32	0.74	1.49	0.72[a]
	1974	0.2	1.4	3.2	10.9	6.0[b]	0.29	0.78	1.46	4.06	1.21[b]
	1975	0.6	5.0	6.8	13.5	5.5[b]	0.12	1.84	3.90	7.44	2.55[b]
	1976	1.5	6.3	15.4	32.0	4.5[b]	1.42	6.15	8.50	22.60	6.85[b]
	1977	0.8	11.2	32.3	47.1	9.9[b]	1.94	5.60	15.52	28.71	5.97[b]
	1978	0.8	9.0	17.3	20.0	6.8[b]	1.91	7.20	16.94	33.70	7.63[b]
	1979	0.4	7.7	21.2	33.8	11.5[b]	3.66	10.30	20.60	37.00	5.75[b]
Zn	1970	58	85	138	212	82[b]					
	1971	28	95	158	259	43[b]					
	1972	56	139	253	381	136[b]					
	1973	60	113	223	328	77[b]	15	34	43	80	33[b]
	1974	59	122	193	293	104[b]	13	43	51	72	9[b]
	1975	38	122	193	281	45[b]	21	74	93	121	16[b]
	1976	66	177	300	391	81[b]	33	109	124	191	42[b]
	1977	71	202	335	454	88[b]	33	81	132	200	41[b]
	1978	73	139	216	308	63[b]	48	114	201	317	72[b]
	1979	59	130	247	330	61[b]	65	100	197	346	67[b]

[a] $0.05 \geq P \geq 0.01$. [b] $0.01 \geq P \geq 0.001$.

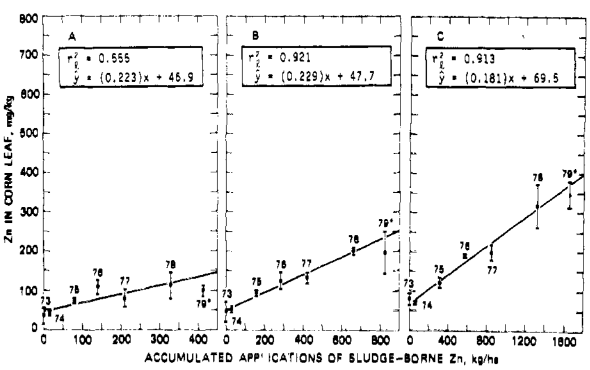

Figure 9. Concentrations (dry weight) of Zn in corn leaves from plots of strip mine spoil treated with 0.25-maximum (A), 0.50-maximum (B), and maximum (C) loading rates of sewage sludge relative to accumulative amounts of Zn applied as a constituent of sludge during each of seven successive growing seasons. (*) Lower than expected Zn concentrations may have been due to an exceedingly low plant population.

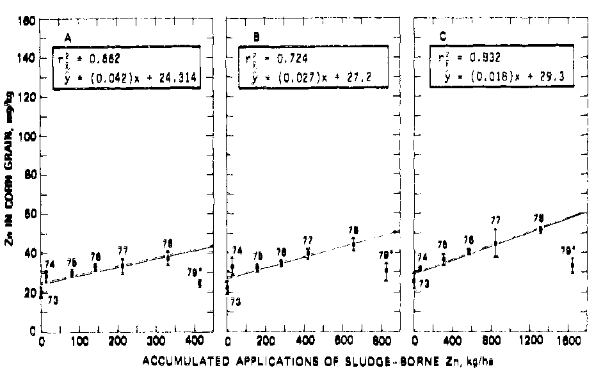

Figure 10. Concentrations (dry weight) of Zn in corn grain from plots of strip mine spoil treated with 0.25-maximum (A), 0.50-maximum (B), and maximum (C) loading rates of sewage sludge relative to accumulative amounts of Zn applied as a constituent of sludge during each of seven successive growing seasons. (*) Lower than expected Zn concentrations may have been due to an exceedingly low plant population.

from the two center rows. Differences in the method of sampling during the last year may have been the reason Zn concentrations in leaves were significantly ($P \leq 0.01$) increased at the 0.50-maximum and maximum cumulative sludge-borne Zn applications (Figure 9B,C) but not at the 0.25-maximum applications (Figure 9A). Grain Zn concentrations were increased by only the maximum sludge applications (Figure 10C). When 1979 data were excluded from the analysis, Zn concentrations in leaves and grain from other treatments were also significantly ($P \leq 0.05$) increased by cumulative applications (Figures 9A and 10A,B).

Mean pH values for 0–15- and 15–30-cm depths (Table V) show that higher sludge loading rates tended to depress the pH of the Blount soil but not of the strip mine spoil. With the maximum sludge loading rate, the Blount soil surface pH had decreased to a value of 5.0 by the fourth year of annual sludge applications. After Blount plots were individually treated with fine ground limestone, the pH of the surface (0–15 cm) layer reached its highest value the following year and then gradually declined. The pH of subsurface zones in Blount was not affected by either sludge or limestone applications. Throughout the 76-cm sampling depth of calcareous, silty clay loam, strip mine spoil, the pH varied from 7.2 to 8.1 and was unaffected by sludge applications.

Table VI shows that when soil samples were collected from Blount after the third year of sludge applications, maximum loading rates had already significantly increased total N and organic C contents in Blount soil. However, by the seventh year organic matter losses resulting from microbial degradation were about equal to amounts added as constituents of sludge. Increases of total N and organic C contents in the surface of strip mine spoil occurred only after high rates of sludge had been applied for 2 years. After the fifth year, further applications of sludge did not increase total N and organic C concentrations in strip mine spoil. Even though equivalent amounts of sludge were annually applied on maximum-treated plots at the two sites, apparent equilibrium organic matter contents in strip mine spoil were established at levels less than half of those in Blount soil. Maximum and 0.50-maximum sludge applications had significantly ($P \leq 0.05$) increased the cation-exchange capacity (CEC) of Blount by 2.7 and 1.3

Table IV. Cadmium and Zinc Concentrations in Corn Grain Grown on Blount Silt Loam and Strip Mine Spoil, with and without Repeated Applications of Sewage Sludge

		mg/kg (dry wt) for sludge irrigation rates									
		0 mm	6.4 mm	12.7 mm	25.4 mm		0 mm	6.4 mm	12.7 mm	25.4 mm	
analyte	year	Blount silt loam				LSD[a]	strip mine spoil				LSD[a]
Cd	1970	0.30	0.60	0.79	1.00	0.02					
	1971	0.14	0.70	0.65	0.92	0.40					
	1972	0.16	0.45	0.83	1.10	0.52					
	1973	0.08	0.15	0.35	0.61	0.06	0.06	0.12	0.12	0.21	n.s.[b]
	1974	0.09	0.18	0.40	0.81	0.22	0.06	0.12	0.18	0.24	0.06
	1975	0.06	0.17	0.28	0.51	0.08	0.08	0.16	0.21	0.38	0.09
	1976	0.09	0.26	0.42	0.84	0.13	0.06	0.14	0.46	0.54	0.28
	1977	0.07	0.25	0.60	0.92	0.22	0.08	0.21	0.46	0.78	0.19
	1978	0.09	0.18	0.46	0.68	0.20	0.16	0.28	0.41	0.83	0.12
	1979	<0.06	0.25	0.43	0.72	0.20	0.15	0.29	0.60	1.12	0.53
Zn	1970	32	40	50	65	15					
	1971	24	37	36	53	10					
	1972	22	29	40	50	13					
	1973	29	37	51	58	6	15	19	22	26	6
	1974	28	37	46	56	7	19	28	33	32	6
	1975	24	36	44	53	6	18	29	32	37	5
	1976	33	42	54	62	9	20	32	35	41	5
	1977	30	43	50	62	9	25	33	39	45	10
	1978	30	39	50	58	6	27	37	44	51	7
	1979	26	42	49	49	9	20	24	30	33	6

[a] Least significant difference at $P \leq 0.01$. [b] n.s., not significant.

Table V. Average pH Values at Two Depths of Blount Silt Loam and Strip Mine Spoil, with and without Applications of Sewage Sludge

		pH for sludge irrigation rates									
		0 mm	6.4 mm	12.7 mm	25.4 mm		0 mm	6.4 mm	12.7 mm	25.4 mm	
depth, cm	year	Blount silt loam				LSD	strip mine spoil				LSD
0-15	1971	5.6	5.9	5.4	5.2	n.s.[c]					
	1972	5.6	5.7	5.2	5.0	n.s.					
	1973	5.6	5.8	5.6	5.3	n.s.	7.8	7.7	8.0	8.0	n.s.
	1974	6.3	6.8	6.4	6.2	n.s.	8.0	8.0	8.1	8.1	n.s.
	1975	7.1	7.2	6.8	6.6	n.s.	7.8	7.7	7.6	7.5	n.s.
	1976	7.0	7.0	6.8	6.4	0.4[a]	7.7	7.7	7.8	7.6	n.s.
	1977	6.4	6.5	6.1	6.0	n.s.	7.2	7.5	7.0	7.3	n.s.
	1978	5.9	6.6	6.1	5.7	0.6[b]	7.3	7.5	7.4	7.2	n.s.
	1979	6.3	6.3	6.1	6.1	0.5[a]	7.8	7.8	7.6	7.5	n.s.
15-30	1974	5.2	5.2	5.3	5.0	n.s.	7.9	7.9	7.8	7.8	n.s.
	1975	6.1	6.4	6.0	5.8	n.s.	8.2	8.1	8.0	8.0	n.s.
	1976	5.9	6.2	6.0	5.7	n.s.	7.8	7.8	7.8	7.4	n.s.
	1977	4.6	5.2	4.9	5.0	n.s.	7.7	7.6	7.6	7.5	n.s.
	1978	5.9	6.4	5.9	5.8	n.s.	7.2	7.5	7.4	7.4	n.s.
	1979	5.8	6.2	5.8	5.6	0.4[a]	8.0	7.9	7.8	7.7	n.s.

[a] $0.05 \geq P \geq 0.01$. [b] $0.01 \geq P \geq 0.001$. [c] n.s., not significant.

mequiv/100 g, respectively, by the last year of the study (Table VI). Only maximum sludge application rates had increased the CEC in strip-mine spoil, but the increase of 3.0 mequiv/100 g was comparable to that in the Blount soil where twice as much sludge had been applied.

DISCUSSION

Application rates rather than soil contents of metals were used in the several regression analyses because this has been the usual practice in analyzing data from fertility studies. But more importantly, regulations governing the disposal of sewage sludges on agricultural lands are based on annual and cumulative application rates (U.S. Environmental Protection Agency, 1979). Because cumulative sludge-borne metal applications had a relatively unimportant effect on plant metal contents in the Blount soil study, data for years and rates were combined for determining annual loading rate effects. But the significant annual effect would have masked the lack of a significant cumulative effect if all loading rates had been combined in the determination of the latter. Only Zn concentrations in leaves from 0.25- and 0.50-maximum sludge-treated Blount plots were significantly affected by repeated application of sludge-borne Cd and Zn. In contrast to data from the Blount study, the statistically significant regression of cumulative loading rates with concentrations of Cd and Zn in plant tissues from strip mine spoil prevented assessment of the effect of annual loading rates. As may be seen in Figures 7–10, the regression slopes for a particular metal concentration in either leaves or grain from spoil were about the same regardless of loading rates. Thus, the data from three sludge loading rates on strip mine spoil could have been combined but are presented separately for comparison with data from Blount soil where relationships were different.

The impact of sludge-borne Cd and Zn on plant composition was greater on calcareous strip mine spoil than on acidic Blount soil. At the beginning of the last year of the study the maximum sludge-treated surface of Blount soil and strip mine spoil had pHs of 6.1 and 7.5, respec-

Table VI. Total N and Organic C Concentrations and Cation-Exchange Capacities (CEC) for Blount Silt Loam and Strip Mine Spoil (0-15-cm Depth), with and without Repeated Applications of Sewage Sludge

		sludge irrigation rates									
		0 mm	6.4 mm	12.7 mm	25.4 mm		0 mm	6.4 mm	12.7 mm	25.4 mm	
analyte	year	Blount silt loam				LSD[a]	strip mine spoil				LSD[a]
		% (Dry Weight)									
N	1971	0.11	0.12	0.13	0.16	0.03					
	1972	0.11	0.14	0.18	0.22	0.03					
	1973	0.11	0.14	0.16	0.20	0.02	0.05	0.04	0.05	0.06	n.s.
	1974	0.14	0.14	0.18	0.28	0.04	0.06	0.05	0.05	0.05	n.s.
	1975	0.13	0.17	0.20	0.31	0.05	0.05	0.05	0.07	0.11	0.04
	1976	0.14	0.14	0.18	0.23	0.04	0.07	0.08	0.09	0.12	0.02
	1977	0.11	0.15	0.21	0.27	0.06	0.07	0.08	0.11	0.14	0.02
	1978	0.13	0.15	0.20	0.28	0.03	0.07	0.08	0.11	0.14	0.02
	1979	0.14	0.17	0.19	0.29	0.04	0.06	0.09	0.11	0.15	0.02
LSD[a]		n.s.[b]	0.01	0.05	0.03		0.01	0.01	0.01	0.03	
organic C	1971	1.34	1.50	1.62	1.94	0.27					
	1972	1.37	1.64	2.04	2.51	0.30					
	1973	1.30	1.56	1.78	2.32	0.20	0.12	0.16	0.18	0.17	n.s.
	1974	1.48	1.74	2.08	3.15	0.47	0.25	0.27	0.30	0.33	n.s.
	1975	1.34	1.85	2.29	3.38	0.37	0.24	0.30	0.52	0.94	0.48
	1976	1.69	1.77	2.28	2.83	0.49	0.33	0.41	0.52	0.76	0.16
	1977	1.34	1.77	2.30	3.03	0.16	0.41	0.48	0.75	1.36	0.18
	1978	1.37	1.68	2.29	2.90	0.45	0.33	0.59	0.94	1.22	0.23
	1979	1.38	1.87	2.60	3.36	0.19	0.40	0.60	0.81	1.36	0.26
LSD[a]		0.20	0.17	0.21	0.50		0.09	0.12	0.14	0.33	
		mequiv/100 g									
CEC	1979	10.9	10.8	12.2	13.6	1.2	12.6	13.8	13.9	15.6	1.9

[a] Least significant difference at $P \leqslant 0.05$. [b] n.s., not significant.

tively. Leaf- and grain-Cd concentrations were respectively 33.8 and 0.78 mg/kg on Blount soil as compared to 37.0 and 1.12 mg/kg on strip mine spoil. Cumulative sludge-borne Cd applications were 157 kg/ha on Blount and 111 kg/ha on spoil. This comparison shows that neither pH nor cumulative sludge-borne Cd applications were dominant factors in controlling the availability of Cd for uptake by corn. The most important factor affecting uptake during the last year was the amount of sludge-borne Cd applied during the previous growing season, which was 19 and 24 kg of Cd/ha on Blount soil and strip mine spoil, respectively. Most of the year to year variations in leaf- and grain-Cd concentrations from the Blount study were due to differences in amounts of Cd applied as a constituent of sludge during the previous growing season. For example, maximum sludge-treated Blount plots received comparable sludge-borne Cd loading rates of 20 and 19 kg/ha during the years previous to 1971 and 1979, respectively. These annual loading rates were used in the linear regression equations (Figure 1) to predict leaf- and grain-Cd concentrations for comparison with measured amounts. Calculated leaf-Cd concentrations were 27.6 mg/kg for 1971 and 26.4 mg/kg for 1979 as compared to measured concentrations of 25.4 and 33.8 mg/kg, respectively. Calculated grain-Cd concentrations were 0.98 mg/kg for 1971 and 0.86 mg/kg for 1979 as compared to measured concentrations of 1.10 and 0.68 mg/kg, respectively. As a result of liming, soil pH was 0.9 unit higher in 1979 than 1971 (Table V) and cumulative sludge-borne Cd had been increased by more than 3-fold by the last year of the study (Table I). In the presence of rather extreme differences in pH and cumulative loading rates, amounts of sludge-borne Cd applied during the previous year were adequate for predicting Cd levels in corn tissues from Blount plots. However, these regression equations cannot be used to predict Cd levels in other corn hybrids grown on the same sludge-amended soil type (Hinesly et al., 1982a) nor for the same hybrid grown on a different soil, as evident by the differences between results from Blount soil and calcareous strip mine spoil materials.

In the pH range of productive agricultural soils, pH adjustment will affect sludge-borne Cd uptake by most field crops to only a minor extent. Jones et al. (1975) found that soil pH interacted significantly with sludge-borne soil Cd contents to affect corn leaf-Cd concentrations in one of the two years in which data were collected. The highest grain-Cd concentrations occurred at a soil pH of about 6. In a study involving four different soil types where natural pHs ranged from 5.71 to 8.25, Street et al. (1977) found that uptake of Cd by corn was less from the most acid soil that also had the highest organic matter content. Corn plants grown on soils amended with a Cd salt ($CdSO_4$) took up more of the metal than when Cd was supplied as a constituent of sludge. Jamison (1944) presented data showing that a minimum solubility of zinc phosphate occurred at a pH near 7 in a fine sand characterized by a high organic matter content. Saeed and Fox (1977) observed a linear decrease in the solubility of Zn with pH in acid soils up to pH 7, but as the system exceeded neutrality, concentrations of Zn increased in solutions from soils with high contents of organic matter. They assumed that the increased solubility of Zn in the alkaline pH range was due to the dispersion of organic matter containing complexed Zn. McBride and Blasiak (1979) found a similar relationship between soil pH and the fixation of Zn in nonexchangeable forms in a silt loam soil. Zinc concentrations in soil solutions decreased as soil pH increased from 5 to 7, but above pH 7 Zn concentrations in solutions increased. They too attributed the increased Zn concentrations in solutions from soils having pH values higher than 7 to dispersion of organic matter by the strong alkali [$Ca(OH)_2$] used to adjust soil pH. They reasoned that $Ca(OH)_2$ was more effective than $CaCO_3$ in reducing the bonding forces of organically complexed Al. However, with the use of $CaCO_3$ they were unable to attain a pH higher than 7.5 in their soil system.

Untreated Blount and spoil had similar CEC's of 10.9 and 12.6 mequiv/100 g, respectively. Cumulative maximum sludge applications significantly increased CEC's to 13.6 and 15.6 mequiv/100 g in Blount and spoil, respectively. Thus, CEC's were not so different between the two sites as to attribute the differences in plant uptake of cumulated sludge-borne Cd and Zn to this factor. Furthermore, Hinesly et al. (1982b) showed that soil CEC had no effect on Cd uptake by corn when the metal was supplied as a constituent of sludge.

The failure of the higher pH of strip mine spoil to control the uptake of Cd and Zn by corn below levels in plants grown on Blount soil was perhaps due to the amounts of organic matter, P, and Fe concomitantly applied with the sludge-borne metals. In maximum sludge-treated plots, organic C contents were increased from 1.3 to 3.1% in Blount soil and from 0.3 to 1.3% in strip mine spoil where amounts lost each year were about equal to inputs. In the same plots P contents were increased from 400 to 3910 mg/kg in Blount soil and from 619 to 2470 mg/kg in strip mine spoil. The digested sludge applied in these studies contained 4.0–5.0% Fe on a dry weight basis. Native concentrations of Fe in the surface layer of Blount soil and strip mine spoil were slightly less than 2.0% and higher than 3.0%, respectively. Significant increases in total Fe contents attributable to sludge applications were not observed, but 0.1 N HCl extractable Fe was increased in the plow layer of the Blount soil.

Fulvic acids extracted from anaerobically digested sludge, the most important water-soluble fraction affecting metal reactions in soil solutions, have been shown to be similar to those extracted from soils (Sposito et al., 1976). Cavallaro and McBride (1980) showed that Cd concentrations in solutions of water-soluble organic matter, extracted from A horizons of two different soil types, were affected very little by change in pH. In solutions that contained 86, 20, and 6.6 mg/L purified humic acid and concentrations of Cd that ranged from 0.1 to 1.0 mg/L, Gardiner (1974) found that the ratios of complexed to uncomplexed Cd were independent of Cd concentration and slightly affected by pH over a range of 6.0–8.5. He concluded that the fraction of humic material responsible for most of the complexation was a strong chelating ligand that was relatively unaffected by pH changes. Riffaldi and Levi-Minzi (1975) presented data showing that a humic acid material extracted from a rendzina soil adsorbed increasing amounts of Cd as pH was increased, but most of the adsorption occurred below a pH of 4. About 50% of the Cd adsorbed by the humic acids was in an exchangeable form and the remainder in coordination complexes. Complexing of Cd and Zn by the humic acids that cumulated as a result of sludge applications may have been one reason why these metals were as available for uptake by corn on calcareous mine spoil as on acid Blount silt loam.

Stanton and Burger (1967, 1970) presented evidence that Fe and Al oxides, in the presence of phosphate ions, fix Zn in forms unavailable to plants. They postulated the bonding of Zn ions between two polyvalent phosphate ions (HPO_4^{2-}) adsorbed on the surface of Fe and/or Al oxides. Considering the ionization of hydrogen ions from phosphate, the theory was consistent with their findings that phosphated Fe oxides commenced to absorb Zn at a pH of 5.25 and increased up to a pH of about 7.25. Above pH of about 7.6, Zn adsorption decreased, probably as a result of decreased P adsorption and the formation of amine and/or hydroxide Zn complexes. The reaction was specific for Zn to the extent that Ca, Mg, K, and NH_4^+ ions did not interfere with its adsorption.

Reports by several researchers suggest that amorphous Fe, Al, Mn, and Si oxides may be the final adsorbents of transition and heavy metal in soils and sediments (Hodgson, 1963; Jenne, 1968; Vuceta and Morgan, 1978). In an examination of eight Manitoba soils, Kalbasi and Racz (1978) found that extractable (HNO_3 plus $HClO_4$) native Zn concentrations were highly correlated with the amorphous and organic complexed Fe and Al in soils but not with Mn. In a review article, Greenland (1971) concluded that when Fe and Al hydroxides are polymerized at clay surfaces, they provided opportunities for strong bonding between clay and organic materials. Hydrous oxides have more extensive and reactive surface areas than clay minerals and the adsorption of organic matter tends to preserve them in a noncrystalline form. Parfitt et al. (1977) found that fulvic acid was more strongly adsorbed on goethite than on gibbsite at a pH of 6 to 6.5, but adsorption on goethite was reduced to a greater extent by higher pH values. Results from studies conducted by Davis and Leckie (1978) suggest that metal ions may have a higher affinity for coordination with chelating groups of adsorbed fulvic or humic acids than with oxide surface sites.

Zinc and Cd have been shown to chemisorb on carbonate surfaces and precipitate as carbonate minerals in calcareous soils (Jurinak and Bauer, 1956; McBride, 1980). Both effects should reduce activities of Zn and Cd in soil solutions and uptake by plants growing on calcareous soils relative to acid soils. But the antithetical results presented here for sludge-borne Zn and Cd, especially for repeated annual applications on calcareous strip mine spoil, suggest that other constituents of sewage sludges alter chemical processes in soils to such an extent that liming of soils may provide little protection against the cycling of these metals in food-chain crops.

Corn plants growing on strip mine spoil exhibited moisture stress symptoms at some stage of growth every year, and this undoubtedly was the reason for lower yields as compared to those from Blount silt loam plots. But concentrations of Cd and Zn in plant tissues were probably not appreciably affected by lower yields. Jastrow and Koeppe (1980) reported that corn plants growing in nutrient solutions containing various levels of poly(ethylene glycol) (an osmoticant) took up less Cd with increasing severity of the water stress condition. Where we applied equivalent amounts of digested sewage sludge and planted the same corn hybrid on silt loam and loamy sand soils in adjacent field lysimeter plots for 5 years, grain and stover yields were often markedly lower on the latter soil type. Due to its low water holding capacity the loamy sand used in the study is not generally planted to row crops in Illinois. However, Cd and Zn concentrations in corn plant tissues from the different soil types were not significantly different (Hinesly and Hansen, 1978). Thus, the available information suggests that the high Cd and Zn concentration in corn plant tissues produced on sludge-amended strip mine spoil cannot be attributed to low biomass production.

Registry No. Cadmium, 7440-43-9; zinc, 7440-66-6; phosphorus, 7723-14-0.

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Chymotrypsin Inhibitor Activity in Winged Beans (*Psophocarpus tetragonolobus*)

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Chymotrypsin inhibitor activity of winged beans (*Psophocarpus tetragonolobus*) was investigated. Extractability of the inhibitors by aqueous solution increases with increasing pH. The chymotrypsin inhibitor activity in winged bean meal was extremely resistant to dry heat treatment. Prolonged boiling (60 min) of the bean was required to destroy the inhibitor activity. Autoclaving is more effective in destroying the chymotrypsin inhibitor activity in the bean meals: 20 min of autoclaving at 120 °C, 1.05 kg/cm^2, generally destroyed more than 90% of the inhibitor activity. There are some varietal variations in the thermal stability of winged bean chymotrypsin inhibitor activity. The chymotrypsin inhibitor activity in the winged bean meal extract is heat labile.

The winged bean, *Psophocarpus tetragonolobus*, is a tropical legume that shows exceptional promise as a food crop for the humid tropics (National Academy of Sciences, 1981). It is also known as four-angled bean or kacang botol in Malaysia. All parts of the winged bean plant are edible and highly nutritious. The mature seed is very similar in composition to the soybean and is of comparable biological value (Claydon, 1975; National Academy of Sciences, 1981).

As with many legume seeds, the mature seed of the winged bean contains a variety of toxic factors (Jaffe and Korte, 1976; National Academy of Sciences, 1981; Tan et al., 1982), including proteinase inhibitors such as chymotrypsin and trypsin inhibitors. It has been established that proteinase inhibitors cause pancreatic hypertrophy in rats, chicks, and mice (Nesheim and Garlich, 1966; Rackis, 1965; Liener and Kakade, 1980) and growth inhibition has been observed in animals fed proteinase inhibitors at high levels (Liener et al., 1949; Ham et al., 1945; Westfall and Hauge, 1948). The antinutritional effects of raw winged bean seed such as growth inhibition, pancreatic hypertropy, and ultimately death are reported to be more deleterious than those of raw soybean (Jaffe and Korte, 1976).

Early studies on the winged bean have indicated the presence of trypsin inhibitor activity (Sohonie and Bhandarkar, 1954). Subsequently, several trypsin inhibitors and a chymotrypsin inhibitor were isolated and purified from winged bean (Kortt, 1979, 1980; Tan et al., 1979; Chan and De Lumen, 1982a). The chymotrypsin inhibitor isolated by Kortt (1980) has a molecular weight of approximately 21 000 and is a stable protein. The inhibitor resists digestion with pepsin at pH 2.0 (Kortt, 1981).

In this paper, we report the results of our investigation on the assay and thermal stability of chymotrypsin inhibitor activity in six varieties of winged beans grown locally.

MATERIALS AND METHODS

Materials. Winged beans of varieties 207, 046, 185, 100, 141, and 095 were grown locally at the experimental farm of Agricultural University of Malaysia, Serdang, Selangor. Soybeans were obtained from local commercial outlets. Winged bean meals were prepared by grounding the mature beans manually with a mortar and pestle. The fine powder obtained was stored below 0 °C in a glass container before use. Bovine α-chymotrypsin and *N*-benzoyl-L-tyrosine-*p*-nitroanilide (BTPNA) were purchased from

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