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HEAVY-METAL ACCUMULATION IN CROPS GROWN ON SEWAGE SLUDGE AMENDED WITH METAL SALTS

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KEY WORDS

Cadmium Chromium Copper Crops Heavy-metal tolerance/interaction Lead Nickel Sewage sludge Toxicity Trees Vegetables

ABSTRACT

Poplar (*Populus euramericana* 'Robusta'), oats (*Avena sativa* L. 'Leander'), maize (*Zea mays* L. 'Ona 36'), English ryegrass (*Lolium perenne* L.), butter head lettuce (*Lactuca sativa* L. 'Reskia'), spinach (*Spinacia oleracea* L. 'Subito') and French beans (*Phaseolus vulgaris* 'Prelude'), were grown in pots with pure sewage sludge (pH 6.7), amended with Cd, Cr, Cu, Ni, Pb and Zn acetates, either added singly or in combination, to study metal effects on plant growth and metal uptake. Phytotoxic metal doses varied with metal and plant species, increasing in the order Cd < Ni < Cu < Zn < Cr and Pb. The threshold dose of toxic metals applied in combination was generally lower than that of metals given singly. Addition of Cd, Ni and Zn was clearly reflected in the respective plant concentrations. This was much less so for Cu, whereas Cr and Pb concentrations were not affected in most plant species. Critical plant (leaf) metal concentrations were lower for metals applied in combination than for single metals. Because of such phenomena the use of critical levels as a diagnostic tool for determining potential multiple metal toxicity is limited.

INTRODUCTION

In densely populated and industrialized countries, disposal of waste materials containing heavy metals presents an ever increasing problem. Injudicious applications to soil give rise to accumulation of heavy metals in the topsoil, adversely affecting plant growth and crop quality. One of the major concerns is heavy-metal enrichment in edible parts of metal-tolerant crops, creating a hazard to animal and human health.

According to guidelines in The Netherlands [7], farmers should only use domestic sewage sludges containing less than 2000 mg Zn, 500 mg Cr, Cu and Pb, 50 mg Ni, 10 mg Cd and Hg, per kg dry-matter. Moreover, applications have to be limited to 2 (metric) tons/ha annually on arable land and 1 ton/ha on grassland. Sewage sludges of non-domestic origin not meeting the standards for heavy-

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metal concentrations are unfit for application on cultivated land and should be disposed on wasteland.

The present study was part of a research project on the possibilities of utilizing discarded sewage sludge contaminated with heavy metals as a substrate for hardwoods like poplar (Populus), alder (Alnus) and birch (Betula). Hardwoods were found to exhibit phytotoxic effects on such growing media which could not be identified and traced to single heavy metals. In attempting to unravel the effects of individual metals, it was thought convenient to use sewage sludge of domestic origin with a low level of heavy-metal contamination as a basic substrate for growing crops and to enrich it with various metals given separately and in combination. By this approach it was also hoped to gain some insight into possible interactions between heavy metals as to plant uptake and phytotoxicity. The results of this work are reported here.

MATERIALS AND METHODS

Poplar (*Populus euramericana* 'Robusta'); poplar and oats (*Avena sativa* L. 'Leander'); maize (*Zea mays* L. 'Ona 36'); English ryegrass (*Lolium perenne* L.) and butter head lettuce (*Lactuca sativa* L. 'Reskia'); spinach (*Spinacia oleracea* L. 'Subito') and French beans (*Phaseolus vulgaris* L. 'Prelude') were grown successively in pots placed in a glasshouse. Growth periods were 15, 2.5, 4, 4.5, 1.5, 1.5 and 2 months, respectively, for poplar (2 seasons), oats, maize, grass (3 cuts), lettuce, spinach and beans. During the 5 growing seasons maximum (day) temperatures varied from 15 to 35°C and (nocturnal) minima from 10 to 20°C. Relative humidity varied from 30 to 70% during the day, and from 70 to 90% during the night. Daylength ranged from 13 to 16.5 hours.

The polythene pots (10 liter) contained 4.3 kg sewage sludge on a dry-weight basis. Reagent grade NH_4NO_3, KNO_3, K_2HPO_4 and $MgSO_4.7H_2O$ were added at rates of 0.7 g N, 0.45 g P, 1.2 g K and 0.3 g Mg per pot for oats, grass (each cut), lettuce and French beans, and at somewhat higher rates of N (1.5 g) and K (2.1 g) for poplar (twice), maize and spinach. This was done some weeks prior to planting or sowing per pot. Test plants were established in pots as follows: poplar, 2 cuttings; oats, seeded directly; maize, 1 seedling; grass, seeded directly; lettuce, 3 seedlings; spinach, seeded directly; beans, 3 seedlings. After harvesting each of the successive crops, substrates of identical treatment (5 replicates) were mixed thoroughly and root residues were removed. A layer of pure sand had to be placed at the bottom of each pot before refilling it, to make up for losses in sludge substrate. Pots (without drain holes) were watered daily with deionized water to 60% of field capacity.

The growth substrate consisted of primary plus activated, anaerobically digested and sand bed dried sewage sludge with the following characteristics: pH (H_2O), 6.7; organic matter, 44; N, 4.7; P, 0.7; and $CaCO_3$, 3.6 g/100 g; Cd, 8.6; Cr, 235; Cu, 1070; Fe, 26,500; Mn, 1080; Ni, 50; Pb, 520; and Zn, 2300 mg/kg dry matter. This sewage sludge meets largely the standards set for use in crop production, with the exception of the high Cu level. Metals were applied once as soluble acetates, either separately or in combination. Separately, at rates (in mg/kg substrate) of 100, 200 or 300 Cd; 1000, 2000 or 3000 Cr, Pb and Zn; 500, 1000 or 1500 Cu; 250, 500 or 750 Ni. Combined, at rates (mg/kg substrate) of 50 Cd + 500 Cr + 250 Cu + 125 Ni + 500 Pb + 500 Zn, or 2, 3, 4, and 5 times these amounts, respectively. Metals were added well before planting the first crop (poplar) as acetates to exclude any positive or negative non-metal growth effects. The acetates were found to decompose quickly following incubation of the substrates at 20°C. Apart from an initial drop in pH following addition of the acetates, lasting only one week, pH of the substrates varied little throughout the experimental period.

A randomized block design was chosen for the experiment, with 5 replications (arranged in 5 blocks) per treatment and sub-blocks for separate metal (6) and combined metal plus control treatments (2).

Oats (grains), maize (cobs), lettuce (head) and beans (pods) were harvested when mature. Grass was cut when 20 cm high (3 times), and spinach when 10 cm high. Poplar plants were cut off by the end of the first growing season and new shoots were allowed to develop for some 3 months in the second season. The various harvested plant parts (Tables 1, 2) were oven-dried at 70°C to estimate dry weights. Samples of dried leaves (poplar, grass (second cut), lettuce, spinach), stem + leaves ($1\frac{1}{2}$ month-old maize seedlings), grains (oats) or pods (beans) were ground, wet-ashed ($HNO_3/H_2SO_4/HClO_4$) and analyzed for Cd, Cr, Cu, Ni, Pb and Zn by atomic absorption spectrophotometry.

All data on dry-matter yield were subjected to an analysis of variance.

RESULTS AND DISCUSSION

Dry-matter yields

Judging from the minimum amounts of (individual) metals needed to produce phytotoxic effects, Cd proved the most toxic of the metals investigated, the lowest dose (100 mg/kg substrate) significantly depressed dry weight of sensitive crops like spinach (Table 1). The largest dose (300 mg/kg substrate) reduced dry weights of all crops other than oats. Nickel treatment significantly depressed dry-matter production of French beans at 250, of spinach at 500 and of all other crops, except maize, at 750 mg/kg substrate-applied metal. Significantly toxic doses for Cu were attained at 1000 mg/kg substrate-applied metal for poplar and spinach, and at 1500 mg for oats, grass and French beans. By contrast, maize and lettuce were not affected by Cu. Zinc additions of 2000 mg/kg substrate proved toxic for poplar, lettuce and spinach, whereas 3000 mg Zn was toxic for grass and French beans. The threshold of toxicity was not reached for oats and maize. Dry-matter production of oats was even stimulated by high Zn. Within the range of Cr and Pb applications (1000–3000 mg/kg substrate) neither metal had a detrimental effect on plant growth. The data are not presented in Table 1. The toxic metal doses for plants grown on a pure sewage sludge substrate are considerably higher than those generally reported for soils[17]. This is attributed to the adverse effect of sewage sludge on metal availability[4,13,16], as related to adsorption sites and pH.

Summarizing the above results for single metals, phytotoxic doses inducing a 20% yield reduction (which was significant in each crop species), increased in the order Cd < Ni < Cu < Zn < Cr and Pb, whether expressing the elements on a weight or on a molar basis. This order of toxicity agrees with that reported by Mitchell, Bingham and Page[15] for Cd, Ni, Cu and Zn in lettuce and wheat, and by Kloke und Schenke[11] for Zn, Cr and Pb in grass.

Yield reduction in the various crops was not necessarily accompanied by chlorosis. Chlorosis patterns produced by excess metals are mainly non-specific, with an exception for Ni toxicity in Gramineae species[1]: alternate bands of different chlorosis intensity across the leaves.

Most crops only exhibit adverse effects following treatment with the higher doses of individual metals (Table 1), whereas combinations of metals severely depress growth at half this level or less (Table 2). In most cases the impact on plant performance of combined metals cannot be explained on the basis of mere summation of the separate effects of individual metals. This is clearly shown when comparing dry-matter yields at the second highest rates of single (Table 1) and combined (Table 2) metals. The nature of this metal interaction is generally synergistic, metal effects reinforcing each other.

Table 1. Dry-matter yields of various crops grown on sewage sludge, following treatment with separate metals

Metal added mg/kg substrate*		Dry-matter yield in g/pot (mean of 5 replications)										
		Poplar		Oats		Maize		Grass	Lettuce	Spinach	French beans	
		Leaves	Stems	Grain	Straw	Cobs	Stem/ Foliage	3 cuts			Pods	Leaves
Control**	0	101	76	54	57	133	88	50	23	13.7	44	30
Cd	100	99	77	56	60	128	98	49	24	8.2	42	28
	200	88	64	55	60	120	99	48	22	4.9	43	27
	300	79	53	58	59	109	72	44	20	4.3	39	26
Significance***		x	x	N.S.	N.S.	N.S.	x	xx	xx	xxx	xx	N.S.
Cu	500	91	64	55	58	126	86	49	22	11.2	46	30
	1000	75	57	52	54	141	70	47	23	7.4	41	30
	1500	76	46	41	47	133	88	44	22	5.5	39	29
Significance		x	x	xxx	xxx	N.S.	N.S.	x	N.S.	xxx	xxx	N.S.
Ni	250	84	56	60	59	124	88	51	21	12.2	37	25
	500	92	57	54	56	127	83	46	23	8.7	23	29
	750	36	17	28	41	133	90	36	6	2.0	4	9
Significance		xx	xx	xxx	xxx	N.S.	N.S.	xxx	xxx	xxx	xxx	xxx
Zn	1000	90	59	57	57	133	83	49	21	12.6	40	26
	2000	43	20	63	65	122	91	45	18	10.1	40	24
	3000	6	4	60	70	131	78	40	16	9.0	38	24
Significance		xxx	xxx	N.S.	xxx	N.S.	N.S.	xxx	xxx	xxx	xx	N.S.

* Substrate contained in mg/kg dry substrate: Cd, 8.6; Cr, 235; Cu, 1070; Ni, 50; Pb, 520; Zn, 2300.

** No metals added.

*** Level of significance for metal effects at $P = 0.05$ (x), 0.01 (xx) or 0.01 (xxx);
N.S. = not significant.

Heavy-metal interactions on plant yields are reported by a number of authors, *e.g.* Cd/Pb in maize by Miller, Hasset and Koeppe[14], Cu/Ni and Cu/Zn in beans by Wallace and Romney[20], Cu + Ni + Zn *versus* Zn in lettuce by Maclean and Dekker[13], Cd + Cu + Zn + Pb *versus* Cd in wheat by Sommer und Stritesky[18]. Striking results are presented by Wallace and Romney[20]: excess Cu and Ni caused yields of French beans to decrease by 28 and 3%, respectively, as against 68% for the combined metals.

The data presented here imply that crop tolerance to heavy metals varies with plant species and (combinations of) metal species. However, there is a definite tendency for maize to be rather tolerant and for spinach and poplar to have a relatively low tolerance. The order of sensitivity of plant species to Cd toxicity agrees fairly well with that described by Kloke und Schenke[11]. The finding by Boawn and Rasmussen[2] that cereal crops are rather sensitive to Zn toxicity as compared with leafy vegetables could not be confirmed. Maclean and Dekker[13] found lettuce to be more susceptible to excess Cu, Ni and Zn than maize, which is more in line with the data presented here (Table 2).

Table 2. Dry-matter yields of various crops grown on sewage sludge following treatment with combined metals

Combined metals added (codes*)		Dry-matter yield in g/pot (mean of 5 replications)										
		Poplar		Oats		Maize		Grass	Lettuce	Spinach	French beans	
		Leaves	Stems	Grain	Straw	Cobs	Stem/ Foliage	3 cuts			Pods	Leaves
Control	0	101	76	54	57	133	88	50	23	13.7	44	30
	1	74	47	58	60	128	81	48	21	9.4	35	24
	2	59	37	54	59	142	91	45	20	5.6	37	27
	3	19	8	10	25	100	91	32	16	2.7	10	19
	4	1	1	1	8	105	98	25	12	2.2	3	10
	5	0	0	1	6	94	84	16	2	2.0	0	2
Significance**		xxx	xxx	xxx	xxx	xx	N.S.	xxx	xxx	xxx	xxx	xxx

* Codes 0 = control (untreated)
1 = 50 Cd + 500 Cr + 250 Cu + 125 Ni + 500 Pb + 500 Zn mg/kg substrate
2 = 100 Cd + 1000 Cr + 500 Cu + 250 Ni + 1000 Pb + 1000 Zn mg/kg substrate
3 = 150 Cd + 1500 Cr + 750 Cu + 375 Ni + 1500 Pb + 1500 Zn mg/kg substrate
4 = 200 Cd + 2000 Cr + 1000 Cu + 500 Ni + 2000 Pb + 2000 Zn mg/kg substrate
5 = 250 Cd + 2500 Cr + 1250 Cu + 625 Ni + 2500 Pb + 2500 Zn mg/kg substrate

** Level of significance for metal effects at $P = 0.05$ (x), 0.01 (xx), or 0.001 (xxx);
N.S. = not significant.

Metal concentration

When interpreting the results it should be borne in mind that the experiment had a non-factorial design, which means that the effect of applied metal A on plant concentration of metal B is only demonstrated in the absence of applied B, however in a medium containing 'native' heavy metals.

Cd and Ni (Tables 3, 4) had a strong positive impact on tissue Cd and Ni

Table 3. Cd-concentrations in various crops grown on sewage sludge (8.6 mg/kg Cd) following application of Cd only, or of combined metals (Cd, Cr, Cu, Ni, Pb, Zn)

Crop	Cd concentration in µg/g dry matter								
	Control	Added Cd (mg/kg)			Added combined metals (codes)*				
		100	200	300	1	2	3	4	5
Poplar (leaves)	2.4	21.4	27.7	39.6	13.2	22.3	37.2	76.1	–**
Oats (grain)	0.29	1.68	3.13	4.06	1.31	1.93	3.28	9.70	9.99
Maize (stem/foliage)	0.7	6.7	12.1	18.3	2.9	6.5	14.2	22.5	22.0
Grass (leaves)	0.77	4.01	6.34	9.14	2.52	4.01	5.47	7.55	8.28
Lettuce (leaves)	2.0	17.1	30.9	47.3	14.0	23.6	37.2	49.2	32.6
Spinach (leaves)	1.9	32.6	43.2	44.4	16.7	22.9	27.9	–	–
Beans (pods)	0.07	0.16	0.47	0.72	0.20	0.43	0.43	0.99	–

* Treatment codes: 1 = 50 Cd + 500 Cr + 250 Cu + 125 Ni + 500 Pb + 500 Zn mg/kg substrate. Treatment codes 2, 3, 4 and 5 represent, respectively, 2, 3, 4 and 5 times the amounts applied according to treatment code 1 (see also Materials and Methods).
** Too little material for analysis.

Table 4. Ni concentrations in various crops grown on sewage sludge (50 mg/kg Ni) following application of Ni only, or of combined metals (Cd, Cr, Cu, Ni, Pb, Zn)

Crop	Ni concentration in µg/g dry matter								
	Control	Added Ni (mg/kg)			Added combined metals (codes)*				
		250	500	750	1	2	3	4	5
Poplar (leaves)	1.3	13.6	24.8	53.4	11.0	13.9	44.0	67.7	–**
Oats (grain)	4.8	37.3	73.4	105	34.3	42.8	99.1	169	160
Maize (stems/foliage)	1.2	3.3	7.6	17.7	2.7	3.0	8.2	11.2	14.3
Grass (leaves)	10.6	80.3	176	251	57.2	76.5	216	247	272
Lettuce (leaves)	4.0	16.5	30.6	78.9	8.6	12.3	33.8	49.6	49.5
Spinach (leaves)	0.51	21.6	54.8	–	15.6	24.6	86.0	–	–
Beans (pods)	2.2	31.9	63.0	112	21.7	27.8	69.3	84.5	–

* Treatment codes: See Note Table 3.
** Too little material for analysis.

Table 5. Zn concentrations in various crops grown on sewage sludge (2300 mg/kg Zn) following application of Zn, Cd, Cu, Ni singly, or of combined metals (Cd, Cr, Cu, Ni, Pb, Zn)

Crop	Zn concentration in μg/g dry matter																
	Con- trol	Added Zn (mg/kg)			Added Cd (mg/kg)			Added Cu (mg/kg)			Added Ni (mg/kg)			Added combined metals (codes)*				
		1000	2000	3000	100	200	300	500	1000	1500	250	500	750	1	2	3	4	5
Poplar (leaves)	446	700	1143	2232	359	335	322	529	545	578	380	194	185	542	536	458	570	—**
Oats (grain)	52	66	99	123	57	57	60	60	73	74	51	56	70	69	84	89	187	193
Maize (stems/foliage)	86	137	157	235	110	100	118	113	122	106	132	137	161	139	202	260	277	324
Grass (leaves)	240	365	453	556	259	252	280	295	318	320	212	229	251	303	365	375	405	395
Lettuce (leaves)	182	351	446	571	150	144	136	236	320	328	214	195	97	246	310	345	329	235
Spinach (leaves)	316	400	491	513	279	202	181	344	350	344	300	248	99	344	323	244	195	157
Beans (pods)	35	42	56	61	35	33	34	36	38	42	32	31	37	38	38	37	32	—

* Treatment codes: See Note Table 3.

** Too little material for analysis.

concentrations. Other metals hardly influenced tissue Cd and Ni, unless applied in combination. In the latter case, metal interaction was mainly synergistic in nature.

Zn (Table 5), strongly increased plant Zn. Separate metals, like Cd, Ni and Cu affected plant Zn positively or negatively. The net effect of metal interaction may

Table 6. Cr concentrations in various crops grown on sewage sludge (235 mg/kg Cr) following application of Cr only, or of combined metals (Cd, Cr, Cu, Ni, Pb, Zn)

Crop	Cr concentration in µg/g dry matter								
	Control	Added Cr (mg/kg)			Added combined metals (codes)*				
		1000	2000	3000	1	2	3	4	5
Poplar (leaves)	0.4	0.4	0.5	0.5	0.2	0.7	0.7	–	–**
Oats (grain)	4.0	4.8	4.7	6.6	3.5	5.6	1.0	–	–
Maize (stems/foliage)	1.3	3.1	2.4	2.6	1.7	1.8	2.0	4.0	3.9
Grass (leaves)	6.3	5.8	6.1	5.3	3.2	4.8	5.4	3.4	3.1
Lettuce (leaves)	3.0	6.7	8.3	13.4	2.7	7.1	5.0	12.6	13.3
Spinach (leaves)	0.14	0.05	0.18	0.28	0.21	0.53	0.80	1.01	1.62
Beans (pods)	0.08	0.16	0.29	0.21	0.45	0.13	0.24	0.16	–

* Treatment codes: See Note Table 3.
** Too little material for analysis.

Table 7. Pb concentrations in various crops grown on sewage sludge (520 mg/kg Pb) following application of Pb only, or of combined metals (Cd, Cr, Cu, Ni, Pb, Zn)

Crop	Pb concentration in µg/g dry matter								
	Control	Added Pb (mg/kg)			Added combined metals (codes)*				
		1000	2000	3000	1	2	3	4	5
Poplar (leaves)	0.5	0.8	1.5	1.2	0.7	1.1	1.3	5.3	–**
Oats (grain)	3.8	2.9	2.7	2.3	0.6	0.1	3.3	–	–
Maize (stems/foliage)	0.3	0.4	0.4	0.4	0.2	0.3	0.4	0.8	0.4
Grass (leaves)	4.9	5.2	5.4	5.5	5.3	5.6	6.0	7.3	7.6
Lettuce (leaves)	9.2	10.8	14.1	20.6	10.2	12.6	16.2	24.4	23.9
Spinach (leaves)	0.24	0.91	0.67	0.68	0.39	0.33	2.04	–	–
Beans (pods)	0.23	0.27	0.37	0.30	0.65	0.56	0.17	0.30	–

* Treatment codes: See Note Table 3.
** Too little material for analysis.

Table 8. Cu concentrations in various crops grown on sewage sludge (1070 mg/kg Cu) following application of Cu, Cd or Ni singly, or of combined metals (Cd, Cr, Cu, Ni, Pb, Zn)

Crop	Cu concentration in μg/g dry matter														
	Control	Added Cu (mg/kg)			Added Cd (mg/kg)			Added Ni (mg/kg)			Added combined metals (codes)*				
		500	1000	1500	100	200	300	250	500	750	1	2	3	4	5
Poplar (leaves)	7.1	8.9	8.7	10.1	6.9	7.7	7.3	6.1	4.8	4.7	6.8	6.6	7.0	14.8	–**
Oats (grain)	11.0	13.0	15.6	15.4	12.4	12.9	12.9	11.1	11.6	13.8	14.6	16.2	15.3	–	–
Maize (stem/foliage)	12.7	13.4	14.6	15.2	14.5	13.9	13.4	13.0	13.5	13.4	12.5	14.1	13.9	14.0	13.8
Grass (leaves)	34.9	36.0	35.2	35.3	33.0	30.8	30.1	30.9	29.2	27.6	31.3	32.8	28.3	29.1	28.6
Lettuce (leaves)	16.3	18.2	21.7	23.0	13.0	12.1	12.9	15.8	14.8	12.6	16.5	19.7	18.4	23.5	20.6
Spinach (leaves)	21.0	32.9	49.3	59.3	21.4	18.5	16.3	20.9	19.6	11.0	27.1	27.9	20.5	15.4	11.7
Beans (pods)	7.9	8.7	10.3	11.7	7.1	6.7	6.5	6.5	6.0	7.1	6.8	7.1	7.3	7.6	–

* Treatment codes: See Note Table 3.

** Too little material for analysis.

be antagonistic (spinach) or synergistic (oats), but it may also be nil, when metal effects are additive (maize).

Cr and Pb (Tables 6, 7) only exceptionally had a positive impact on plant Cr and Pb concentrations. In such (accumulating) plant species other metals have little effect on Cr and Pb in plant tissues, unless applied in combination where synergistic phenomena may occur. The high values for lettuce are difficult to explain.

Cu (Table 8) in most plant species only slightly raised plant Cu. Separate metals like Cd and Ni influenced plant Cu to some extent. The effect of combined applied metals was to reduce (antagonism, spinach), in other cases, to increase (synergism, lettuce) plant Cu more than is accounted for by the action of separate metals.

The metal uptake pattern is in line with published data, viz. upon application of the respective metals relatively large increases in plant (leaf) Cd, Ni, Zn, and minor effects on Cu, as shown for lettuce and maize[12], wheat[18]; little rise in plant Pb, as shown in spinach[9], wheat[18]; little influence also on plant Cr, as reported for wheat, spinach and grass[10].

Similar to crop plant yields, metal interactions are described for plant chemical composition: Cd/Pb with grass[3], Cu/Zn with maize[5], Cd + Cr + Cu + Ni + Zn *versus* single metals in soybeans[6]. Maclean[12] found a positive effect of added Cd on plant (lettuce) Zn, but the reverse was not true. This partly agrees with the data presented here, but added Cd was shown to decrease plant Zn (Table 5). According to Haghiri[8] low Zn was found to increase and high Zn to decrease plant Cd (soybean). These examples show that metal interrelationships are rather complex in nature.

Table 9. Leaf metal concentrations associated with a 20% reduction in dry-matter production in various crops, both for metals applied singly and combined

Crop	Concentration in µg/g foliar dry matter							
	Cd concn.		Ni concn.		Zn concn.		Cu concn.	
	Cd added singly	Cd added with other metals	Ni added singly	Ni added with other metals	Zn added single	Zn added with other metals	Cu added singly	Cu added with other metals
Poplar	40	13	30	11	800	540	9	7
Grass	> 9	5	220	130	560	370	>35	30
Lettuce	>47	30	45	20	430	320	>23	20
Spinach	15	10	30	10	460	330	35	25

The design of the experiment does not permit the determination of sharp threshold plant (leaf) concentrations for metal toxicity. However, it is evident that plant (leaf) metal concentrations associated with a 20% yield reduction (which was significant in each crop species) were lower for applied combined than for single metals (Table 9). For instance, poplar growth was reduced at 40 μg/g leaf Cd (Tables 1 and 3), following application of 300 mg/kg Cd singly. For Ni, Zn and Cu, critical leaf values may be established, by interpolation, of 30, 800 and 9 μg/g, respectively, if the metals are added separately. As a contrast, combined metals were detrimental at the first level of application (treatment code 1; Table 2), plant foliage containing 13 μg/g Cd (Table 3), 11 μg/g Ni (Table 4), 540 μg/g Zn (Table 5) and 7 μg/g Cu (Table 8). Apparently none of the latter concentrations in itself is indicative of phytotoxicity, but the combination of concentrations is. It is not possible to unravel the parts played by Cr and Pb in the heavy-metal syndrome, if any, as none of these metals were added in quantities sufficiently high to induce toxicity. Carlson and Rolfe[3] point out that, for threshold Pb and Cd concentrations in some grass species, Pb/Cd interaction may be either additive, synergistic or antagonistic. Because of such phenomena the use of critical levels as a diagnostic tool for determining potential metal toxicity is limited. Moreover, Mitchell *et al.*[15] showed that phytotoxic metal concentrations in lettuce and wheat varied with soil type.

As pointed out before, the effects of combined metals on plant metal concentrations are complex as compared with single metals. However, when metal contents in plant tissues are expressed in absolute amounts (μg) rather than in concentrations (μg/g), the patterns for the various metals become more uniform. This supports findings reported by Strickland, Chaney and Lamoreaux[19] in soybeans. The data for total amounts are not presented here, but they can easily be calculated from Tables 1, 2 and Tables 3 to 8 inclusive. Total amounts of metals in plant tissues were found to be higher for metals added separately than for combined metals, whereas in most cases the opposite holds for concentrations.

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