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Effects of Genotype and Sewage Sludge on Cadmium Concentration in Lettuce Leaf Tissue

Gene T. Yuran[1] and Helen C. Harrison[2]
Department of Horticulture, University of Wisconsin–Madison, Madison, WI 53706

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Abstract. **Leaf Cd concentrations were determined for 60 commercial cultivars and USDA plant introductions of noncrisphead lettuce (*Lactuca sativa* L.). This 2-year study evaluated each genotype in field plots receiving an industrial sludge, a municipal sludge, or no sludge amendment. Genotype and sludge treatment had significant effects on leaf Cd concentration. Although the genotype × year interaction was also significant, the Cd concentrations of some cultivars ('Waldmann's Green' and 'Summer Queen') were consistently low while others (PI 278080, PI 167148, and 'Citation') were consistently high. Plots amended with the industrial sludge had the highest total and available Cd levels, and plants grown on these plots had the highest leaf Cd concentrations, ranging from 3.1 to 11.9 $\mu g \cdot g^{-1}$. Leaf Cd concentrations from plants grown on the municipal sludge-amended plots ranged from 1.4 to 5.5 $\mu g \cdot g^{-1}$ and from 1.2 to 2.5 $\mu g \cdot g^{-1}$ for the control plots.**

One of the primary health concerns of applying sewage sludge as a fertilizer or soil conditioner is the introduction of Cd into the food chain (6, 17, 19). As the soil and plant factors that control Cd uptake and accumulation are identified, crop production practices can be developed to minimize the amount of Cd that enters the food chain. A potential component of these production practices may be the use of cultivars that accumulate little or no Cd.

Varietal differences in Cd accumulation have been found in barley (8), soybeans (4), corn (13), wheat (2, 16), carrots (11), cucumber (12), and lettuce (5, 10, 15), but not in potatoes (9). Except for the work of Hinesly et al. (13) and Boggess et al. (4), these studies have surveyed only a limited genetic base of 3 to 9 cultivars each. May of these studies (4, 13, 15, 18) have not examined the stability of cultivars over time or sludge sources. Finally, some studies used Cd salts in hydroponic solutions or small volumes of soil to screen cultivars, so their conclusions may not be applicable to sewage sludge-amended field plots (5, 18).

This study examined leaf Cd concentrations in 60 commercial cultivars and USDA plant introductions of noncrisphead lettuce. The stability of these genotypes for Cd concentration also was determined over sludge sources and time.

Materials and Methods

Sixty noncrisphead cultivars and USDA plant introductions of lettuce were grown in 1983 and 1984 on sludge-amended plots located at the Horticulture Research Farm in Arlington, Wis., as part of a continuous study initiated in 1981 (12). A split-plot design with 3 replications was used, where sludge type (municipal, industrial, or none) was the whole plot and genotype the subplot. The municipal and industrial sludges were obtained in Madison and Racine, Wis., respectively.

Sludge analyses are provided in Table 1. The municipal sludge was selected for its relatively low heavy metal content and the industrial for its relatively high heavy metal content. Each sludge was applied at a rate of 90 $t \cdot ha^{-1} \cdot year^{-1}$ from 1981 through 1984. Although sludge analyses did vary from year to year (11), relative element rankings within and between sludges remained constant.

Table 1. Analyses of municipal and industrial sludges for 1984.[z]

	Analytical results[y]	
		Sludge type
Constituent	Municipal	Industrial
pH	6.7	7.1
NH_4-N	0	300
ORG-N	16,800	10,700
TOT-N	16,800	11,000
P	8,500	12,200
K	1,700	3,700
Mg	14,200	13,400
Ca	49,000	27,600
Al	5,200	22,700
Mn	328	631
Fe	10,900	34,300
Zn	1,010	1,590
Cd	12	57
Pb	182	292
Cu	193	293
Ni	32	118

[z]Analyses for 1983 are not available.
[y]All values except pH in $mg \cdot kg^{-1}$ on a dry-weight basis.

To increase Cd availability and thereby maximize plant uptake, elemental S was applied at a rate of 1680 $kg \cdot ha^{-1}$ in Spring 1983 to reduce soil pH. Soil pH was recorded each year. In Spring 1985 one soil sample from each sludge type was analyzed at The Pennsylvania State Univ. Soil Testing Laboratory for Cd content and availability. In both years, supplemental fertilizer was applied according to the recommendations of Kelling et al. (15).

During July 1983 and June 1984, seeds of each genotype were planted in 1-m rows with 0.3 m between rows. There was one genotype per row per sludge type per replication. Seedlings were thinned to 8 to 12 mature plants per row. Standard cultural practices were followed, and irrigation was applied as needed.

Two to 3 basal leaves from each of the 8 to 12 plants per row were harvested 7 to 8 weeks after planting and composited for Cd analysis. Leaves were rinsed twice in tap water and once in distilled water, dried at 60°C in a forced-air oven, and ground in a stainless-steel Wiley mill.

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[1]Graduate Research Assistant.
[2]Assistant Professor of Horticulture.

Analysis for Cd followed the procedure of Chaney et al. (7). A 2-g sample of dried leaf tissue was ashed at 480°C for 10 hr. For tissue grown in 1983, 4 ml of concentrated nitric acid were added to the ash and heated until about 0.5 ml of liquid remained. Ten milliliters of 3 N hydrochloric acid were then added, and the mixture was refluxed for 2 hr. For tissue grown in 1984, the ash was dissolved in 10 ml of 3 N hydrochloric acid, and the mixture was refluxed for one hour. The 1984 modification required less time to perform than the 1983 tests, but yielded similar results. All samples were then filtered and diluted to 25 ml. Cadmium concentrations were determined by flame atomic absorption spectrophotometry. Background correction was not used, since all samples contained more than 1 $\mu g \cdot g^{-1}$ Cd on a dry weight basis.

Preliminary analyses of data revealed that the variance in Cd concentration in lettuce leaves increased with the mean; consequently, analyses were performed on log-transformed data (1). Residual plots of the log-transformed data indicated that the variance had been stabilized and that there were no other violations regarding assumptions for analysis of variance. No outliers were found.

Results and Discussion

Soil pH and Cd levels. Soil pH and Cd levels are presented in Table 2. The plot amended with industrial sludge had the highest total and available Cd (9.0 $kg \cdot ha^{-1}$ and a pCd of 12.1). The municipal sludge-amended plot had the next highest levels, and the control plot had the lowest actual and available Cd. The available Cd in the control, municipal, and industrial sludge-amended plots was in the low-normal, normal, and high ranges, respectively (3).

Growth response. In both years, phytoxicity from either sludge was not observed, although viral infections became apparent in some plants after 6 weeks.

Sludge effects. Significant effects of sludge application, which were averaged over the 60 genotypes, were observed on leaf Cd concentrations (Table 2). Plants grown on plots amended with the industrial sludge had the highest leaf Cd concentrations—5.7 and 6.4 $\mu g \cdot g^{-1}$ in 1983 and 1984, respectively. Plants grown on the plots amended with municipal sludge averaged about one-half the Cd found with the industrial sludge, and those grown on the control plots had the lowest Cd concentrations.

The effect of sludge type on leaf Cd concentrations could be explained on the basis of the total and available Cd levels in the soil, e.g., the industrial sludge-amended plots had the highest level of total and available Cd (Table 2), and plants grown on these plots had the highest leaf Cd concentrations. To the extent that increasing total Cd increases available Cd, these results support the findings of Hinesly et al. (13) and Chang et al. (8), who found that as total soil Cd increased, so did the tissue Cd concentrations in corn and barley. Soil Cd levels were varied in those studies by incremental application of the same sludge, while in this study soil Cd levels were varied by similar application rates of different sludges. Although the experiments of this study were not designed to determine the relationship between Cd levels in the soil and those in the plant, one can see that lettuce Cd was not a simple linear function of applied Cd (Table 2).

Genotype effects. Differences in leaf Cd concentrations existed among the genotypes tested (Table 3). However, the significant genotype × year interaction and Spearman's rank correlation coefficient, $r_s = 0.27$ (significant at the 5% but not 1% level) prevent generalizations from being made regarding genotypic performance over years. Instead, the genotypic performance or ranking has to be examined for each year separately (Table 4). Genotypes are listed in Table 4 from lowest to highest Cd concentrations in 1983; consequently, low ranks are associated with low concentrations and high ranks are associated with high concentrations.

The significant genotype × year interaction term resulted from the performance of cultivars such as 'Salad Bowl' and PI 176585, which had high Cd concentrations in 1983 (ranked 56 and 52), but much lower ones in 1984 (ranked 17 and 13). Conversely, PI 278107 (ranked 3) and PI 181946 (ranked 6) had low Cd concentrations in 1983 but high ones in 1984 (ranked 43 and 54). Not all genotypes, however, performed in this manner.

Several genotypes were consistently high or low in both years. Examples of genotypes with high Cd concentrations included PI 278080, PI 344449, PI 167148, and 'Citation', which were ranked 60 and 56, 58 and 51, 55 and 57, and 54 and 53 for 1983 and 1984, respectively. These genotypes were significantly different from genotypes with consistently low Cd concentrations, such as 'Waldmann's Green', which was ranked 2 both years and 'Summer Queen', which was ranked 7 in 1983 and 8 in 1984.

Although significant differences were found among genotypes, many genotypes were indistinguishable from each other statistically. In 1983, for example, PI 278071 had a mean of 2.73 $\mu g \cdot g^{-1}$ Cd and a ranking of 3 and was not different from the succeeding 44 genotypes. The ability to distinguish only the

Table 2. Soil pH and effects of sludge type on Cd levels of soil and of leaf tissue.

Sludge type	pH[z] 1983	pH[z] 1984	pH[z] 1985	Soil Cd ($kg \cdot ha^{-1}$) Total	Soil Cd ($kg \cdot ha^{-1}$) Available[y]	Leaf Cd (dry wt, $\mu g \cdot g^{-1}$)[x] 1983	Leaf Cd (dry wt, $\mu g \cdot g^{-1}$)[x] 1984
Control	6.4 a	4.6 a	4.7 a	0.09	13.52	1.6 a	1.9 a
Municipal	6.7 a	5.5 b	5.8 b	1.10	13.36	2.7 b	2.9 b
Industrial	6.7 a	5.3 b	6.0 b	9.00	12.10	5.7 c	6.4 c

[z]Mean within years separated by Duncan's multiple range test, 5% level.

[y]Availability is expressed as the negative logarithm of ionic activities. The lower the number the higher the availability. Low, normal, and high ranges are 14.5–13.5, 13.5–12.5 and 12.5–11.0, respectively.

[x]Means within years separated by Duncan's multiple range test, P = 5%, on log-transformed data. Data reported as arithmetic means.

Table 3. Analysis of variance of a split-plot design for leaf Cd concentration in lettuce.[z]

Source	df	Mean square
Year	1	2.05
Rep (year)	4	0.69
Sludge	2	140.50**
Sludge × year	2	0.10
Whole plot error	8	2.63
Genotype	62	0.26**
Genotype × year	62	0.15**
Genotype × sludge	124	0.06
Genotype × sludge × year	124	0.08
Subplot error	727	0.07

[z]Data were logarithmically transformed.

**Significant at 1% level. Other sources not significant.

Table 4. Leaf Cd concentrations of 60 noncrisphead lettuce lines.

Cultivar or USDA plant introduction no.	1983 Rank[z]	1983 Cd concn dry wt ($\mu g \cdot g^{-1}$)[y]	1984 Rank[z]	1984 Cd concn dry wt ($\mu g \cdot g^{-1}$)[y]
176587	1	2.24 a	20	3.06 b–i
Waldmann's Green	2	2.50 ab	2	2.96 ab
278071	3	2.73 abc	43	3.65 b–l
178921	4	2.71 a–d	34	3.85 b–l
278095	5	2.83 a–e	44	4.11 b–l
181946	6	2.91 a–e	54	4.45 h–l
Summer Queen	7	2.68 a–f	8	3.41 a–e
222994	8	2.81 a–g	35	3.89 b–l
278081	9	2.75 a–h	19	3.63 b–i
222254	10	2.64 a–h	40	3.79 b–l
Buttercrunch	11	2.78 a–h	5	3.15 a–d
169493	12	2.71 a–h	4	2.74 abc
278107	13	3.00 a–h	49	4.13 e–l
206444	14	2.82 a–h	28	3.44 b–k
169495	15	2.74 a–h	18	3.29 b–i
140398	16	2.74 a–h	1	2.13 a
141680	17	2.87 a–h	23	3.36 b–j
226514	18	2.86 a–i	14	2.89 b–h
177426	19	3.00 a–i	38	3.65 b–l
169496	20	3.13 a–j	15	3.32 b–h
278111	21	3.22 a–j	11	3.14 b–f
175738	22	3.20 a–j	30	3.89 b–l
181882	23	3.08 a–k	27	3.51 b–k
169509	24	2.96 a–k	12	3.04 b–g
263869	25	3.01 a–l	48	3.80 c–l
181883	26	3.17 a–m	58	5.60 klm
172920	27	3.36 a–m	47	3.97 c–l
169501	28	3.28 a–m	41	3.71 b–l
204707	29	3.30 a–m	36	3.65 b–l
278103	30	3.10 a–m	55	4.74 i–l
288244	31	3.30 a–m	9	3.11 b–f
Ruby	32	3.06 a–m	3	3.09 abc
278093	33	3.90 a–m	10	2.84 b–f
176579	34	3.27 a–m	21	3.10 b–j
278101	35	3.53 b–m	29	3.54 b–k
183234	36	3.58 b–m	45	3.59 b–l
Grand Rapids TBR	37	3.62 b–m	6	2.81 a–d
390977	38	3.56 b–m	24	3.31 b–j
164940	39	3.29 b–m	50	4.38 d–l
176594	40	3.40 b–m	22	3.32 b–j
278083	41	3.52 b–m	52	4.44 f–l
278067	42	3.37 b–m	60	6.98 m
Deep Red	43	3.15 c–m	7	3.13 a–e
278108	44	3.44 c–m	42	4.04 b–l
Black Seeded Simpson	45	3.54 c–m	31	3.76 b–l
169514	46	3.54 c–m	25	3.22 b–j
278063	47	3.47 c–n	59	5.14 lm
391601	48	3.96 c–n	16	3.05 b–h
120962	49	3.30 c–n	32	3.82 b–l
171669	50	3.82 d–n	37	4.09 b–l
344074	51	4.10 f–n	26	3.33 b–k
176585	52	3.86 g–n	13	3.12 b–h
167150	53	4.01 g–n	39	3.80 b–l
Citation	54	3.89 h–n	53	5.10 g–l
167148	55	3.94 i–n	57	5.49 j–m
Salad Bowl	56	3.91 j–n	17	3.65 b–i
165063	57	3.87 k–n	46	3.93 b–l
344449	58	4.20 lmn	51	4.27 e–l
344448	59	4.72 mn	33	3.55 b–l
278080	60	5.16 n	56	5.31 i–l

[z]Ranks ordered from 1 = lowest to 60 = highest.
[y]Means within years separated by Duncan's multiple range test, 5% level, on log-tranformed data; however, arithmetic means are reported with Duncan's test results.

most extreme genotypic differences in Cd concentration was not surprising given the variable conditions found in most field screenings. In 1983, the pooled variance for all cultivars was 1.09 and the coefficient of variation was 26.8%. In 1984, the variance was 2.52 and the coefficient of variation was 31.3%. Although significant differences in concentrations might have been reduced if these genotypes had been grown in the relative controlled conditions of a greenhouse or growth chamber, the results could not necessarily be applied to a field situation (5, 18).

The absence of a significant genotype × sludge interaction (Table 3) indicates that the ranking of the genotypes on the basis of their Cd concentration is similar in both sludges and control. Chang et al. (8) also found an insignificant interaction between genotype and rate of sludge application for tissue Cd concentrations of barley. Stability of genotypic performance over various sources or amounts of sludge is desirable, since rankings can be determined with just one sludge type or rate but can then be extrapolated to other types or rates.

Even though the rankings remained constant over the sludge treatments, the absolute concentrations did not. In 1983, the genotypic means ranged from 1.2 to 2.4, 1.9 to 4.4, and 3.1 to 9.3 $\mu g \cdot g^{-1}$ for the control, municipal, and industrial sludge-amended plots, respectively. In 1984, the means ranged from 1.2 to 2.5, 1.4 to 5.5, and 3.7 to 11.9 $\mu g \cdot g^{-1}$ for the control, municipal, and industrial sludge plots, respectively.

Chaney and Feder (see ref. 5) found a similar range for Cd in lettuce of 3.8 to 8.1 $\mu l \cdot g^{-1}$, even though they used 51 fewer genotypes than were analyzed in this study. The variation in lettuce for Cd concentration does not appear to be great, especially when compared to other crops. In corn for example, Hinesly et al. (13) found that 20 inbred lines varied from 2.5 to 62.9 $\mu g \cdot g^{-1}$ Cd in leaves and 0.1 to 3.9 $\mu g \cdot g^{-1}$ Cd in grain.

Given the significant genotype × year interaction seen in this study, the cultivar differences found for Cd concentration in lettuce by other workers (5, 14) may need to be reevaluated to determine their stability over time. The absence of a genotype × sludge interaction indicates that the screening of genotypes for Cd concentration over more than one sludge source probably is unwarranted. Future studies should evaluate these lines in several locations to determine their stability over different soil types and climates.

The identification of lettuce lines that are stable in their tendency to accumulate both low and high levels of Cd now can be used to study the genetic inheritance of Cd accumulation as well as the physiological/anatomical mechanisms responsible for these differences.

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Effect of Excess Boron on Broccoli, Cauliflower, and Radish

L.E. Francois[1]

Agricultural Research Service, U.S. Department of Agriculture, U.S. Salinity Laboratory, Riverside, CA 92501

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Abstract. **The boron tolerance of broccoli (*Brassica oleracea* L. Italica Group), cauliflower (*Brassica oleracea* L. Botrytis Group), and radish (*Raphanus sativus* L.) was determined in large, outdoor sand cultures. Boron treatments were imposed by irrigation with culture solutions that contained 1.0, 4.0, 8.0, 12.0, 16.0, or 20.0 mg B·liter^{-1} for broccoli and cauliflower, and 1.0, 3.0, 6.0, 10.0, 13.0, or 16.0 mg B·liter^{-1} for radish. Relative yield was reduced 1.8%, 1.9%, and 1.4% with each unit (mg·liter^{-1}) increase in soil solution B (B_{sw}) above 1.0, 4.0, and 1.0 mg B·liter^{-1} for broccoli, cauliflower, and radish, respectively. Increasing B_{sw} significantly reduced plant size of all 3 vegetables. Over the B range tested, no leaf injury was apparent for these 3 vegetables.**

Although B is an essential constituent of the soil solution for normal plant growth, the difference between adequate and toxic concentrations may be only a few milligrams per liter (5). Consequently, excess applications of B-containing fertilizers (14) or the use of B-containing irrigation waters (5) can result in toxic levels of B in the soil. The absorption of this excess B by a crop will cause plant injury and yield decline.

Eaton (5) has reported that B is carried to the leaves in the transpirational stream, where it moves from the veins into the interveinal tissue and accumulates at the tip and margins, resulting in leaf necrosis. Data on the metabolic disruption within the plant tissues caused by excess B absorption is still lacking.

Boron tolerance classification for many vegetable crops has been based upon the incidence of B injury and not on the yield decline of the harvested product (5, 13). However, some studies have shown that unless the product is a leafy type vegetable, the occurrence of leaf injury is not a reliable indicator for B tolerance (7, 12). Consequently, reliable quantitative data based on yield for many vegetables are lacking. Therefore, this study was initiated to determine the B tolerance of broccoli, cauliflower, and radish as measured by the yield and quality of the fresh product.

Materials and Methods

Twenty-four sand tanks (2.08 × 0.86 × 0.84 m deep), which contained a coarse river sand, were used in these tests. The sand was washed to remove fine soil particles that could absorb B from the irrigation waters. Each sand tank was irrigated from a 1365-liter reservoir that contained nutrient solutions with different B concentrations. Irrigation waters were surface-applied 3 times each day, with the sand being completely saturated with each irrigation. The applied solutions were collected in corregated polyethylene tile lines located in the bottom of each tank, and returned to the reservoirs by gravity flow.

The irrigation waters contained 2.0 mM $Ca(NO_3)_2$, 1.5 mM KCl, 1.0 mM $MgSO_4$, 0.5 mM $NH_4H_2PO_4$, 0.5 mg·liter^{-1} Fe as chelated sodium ferric diethylenetriamine pentaacetate, 0.25

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[1]Research Agronomist.