Davidonis, G. H., Hamilton, R. H., Mumma, R. O., *Plant Physiol.* **62**, 80 (1978).
Davis, D. G., Wergin, W. P., Dusababek, K. E., *Pestic. Biochem. Physiol.* **8**, 84 (1978).
Feung, C. S., Mumma, R. O., Hamilton, R. H., *J. Agric. Food Chem.* **22**, 307 (1974).
Feung, C. S., Hamilton, R. H., Mumma, R. O., *Plant Physiol.* **59**, 91 (1977).
Miller, C. O., *Modern Methods Plant Anal.* **6**, 194 (1963).
Wang, H. C., Hamilton, R. H., Deering, R. A., in "Biochemistry and Physiology of Plant Growth Substances", Wightman, R., Setterfield, G., Ed., Runge Press, Ottawa, Canada, 1968, p 685.

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Translocation and Accumulation of Seven Heavy Metals in Tissues of Corn Plants Grown on Sludge-Treated Strip-Mined Soil

William J. Garcia,* Charles W. Blessin, Harold W. Sandford,[1] and George E. Inglett

Metal translocation studies for Zn, Mn, Cu, Pb, Cr, Cd, and Hg were conducted for corn (*Zea mays* L.) plants grown on strip-mined soil amended with anaerobically digested sewage sludge (25 dry tons/acre). Differential metal accumulation rates in the seven tissues analyzed showed that generally the highest metal concentrations occur in the leaves and roots and the lowest in the grain and cob. With the exception of Mn and Hg, metal concentrations increased in tissues as a result of sludge application. The greatest increases were for Cd where mean tissue concentrations (ppm) for unamended and sludge-grown conditions respectively were roots: 0.062, 3.63; lower stems: 0.027, 0.204; and leaves: 0.276, 1.52. Metal extractabilities for plant uptake were established for the soil samples adhering directly to the root system of the corn plants studied; these extractabilities were then compared to metal concentrations in the leaf and root tissues.

The use of anaerobically digested sewage sludge as a product to enhance the agricultural productivity of marginal soils currently is receiving wide attention. Studies with marginal agricultural land, which is naturally deficient in nutrients and organic matter, have evaluated sludge application methods and rates involving field corn and pasture plots (Hyde, 1976). However, the reclamation of strip-mined soils (Boesch, 1974) represents a major effort in this area, primarily because extensive strip-mined lands exist now, and it is expected that more areas will be strip mined as the development of coal resources accelerates. Definitive studies are needed to establish if sludge can be used effectively and safely as a soil conditioner and fertilizer for growing crops. The benefits of land application of sewage sludge for growing crops and the potential problems relating to the uptake of metals by these plants, as well as the subsequent incorporation of these metals into the food chain, have been reviewed by Hinesly et al. (1972), Leeper (1972), Chaney (1973), and Page (1974).

The present study involves corn grown in Fulton County in central Illinois on strip-mined soil amended with anaerobically digested sludge by the Metropolitan Sanitary District of Greater Chicago. Sludge had been applied topically to the soil as a liquid at the rate of 25 dry tons/acre (56 metric tons/ha). In a former study (Garcia et al., 1974a), the quality of the harvested grain was assessed and the heavy metal content was established for the three contiguous corn plant tissues—kernels, cobs, and husks—to determine if any hazard might be expected from the grain itself or the plant tissues directly contacting the grain. This study examines the translocation of Zn, Mn, Cu, Pb, Cr, Cd, and Hg from the soil to corn tissues of the total corn plants to establish where each metal may accumulate. The study also examines possible effects of these metals as they enter the food chain.

To make strip-mine soils agriculturally productive by treatment with sludge, crop quality and yield must improve as a direct result of the treatment. The levels of heavy metals translocated from the amended soil to the plant must not be extensive enough (a) to be phytotoxic or (b) to cause the plant to accumulate metals at levels high enough to be hazardous for consumption by animals or humans.

The uptake of metals by plants is complex and is governed not only by the metal content of the soil, but also is influenced greatly by such factors as: metal availability to plants, soil pH, soil organic matter content, and competitive metal interactions. For definitive translocation studies, it is thus important to assess the availability to the plant of each metal from both unamended and sludge-treated soils. Metal availability usually is approximated by various treatments of the soil, either by mild chemical extractions or by metal complexation techniques. The total metal content of the soil must first be established for each metal studied. This preferably is done on the soil adhering to the root system of the selected plants and should include the rhizosphere, where plant–soil interactions occur (Nicholas, 1965). The root zone depth will vary with plant size; larger plants with larger root systems will encompass soil samples at greater depths.

The uptake of the metals can then be demonstrated by analysis of the total plant, where metal concentrations are determined for the major plant tissues. This serves to define metal distribution patterns within the plant and will show where concentrations of toxic elements may have accumulated in the tissues. Such information is useful in determining if specific plant tissues can be utilized se-

Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, Illinois 61604.
[1]Present address: University of Michigan, Ann Arbor, Michigan 48109.

lectively and safely for human consumption and/or animal feeding. Concentrations of the individual metals chemically extracted from the soil are then compared with concentrations of the metals occurring in the tissues of the plant. However, it is difficult to attain good correlative results for most metals with any one extraction solvent. Strip-mined soils would be expected to exhibit considerably different metal availabilities before and after being amended by the sludge. The metals in the strip-mined soils are bound differently than metals in the sludge. In a study with 12 different wastewater sludges (Stover et al., 1976), metal retention on the sludge was measured for Pb, Cu, Zn, Cd, and Ni and was judged to occur by mechanisms that included: ion exchange, sorption, chelation, and precipitation—where the metals were associated as sulfides, carbonates, adsorbed, exchangeable, and organic bound. After the interaction of strip-mine soil with the sludge, it would thus be very difficult to assess how the metals are bound.

MATERIALS AND METHODS

In 1971, two large test plots of strip-mined land, approximately 5 acres each, were prepared, tilled, and cultivated by the Metropolitan Sanitary District of Greater Chicago at their Fulton County strip-mine reclamation site. This area represents formerly productive agricultural land which had been strip-mined for coal and where the original top soil had been displaced. Anaerobically digested liquid sludge, averaging about 5% total solids, was pumped from a holding lagoon and applied topically both before planting of a yellow dent field corn hybrid and several times during the growing season as weather permitted. The soil of one plot received a cumulative amount of 25 dry tons/acre. Metal content of a sludge sample collected at the site was previously reported (Garcia et al., 1974a). The other plot was untreated.

Corn plants were selected in the field plots at the Fulton County site to study the translocation of the seven metals from the soil to the tissues of corn plants of small and large size. The selection was made according to the following criterion: (a) two corn plants must be growing near each other, but each must be of a different size—to observe the effects of different growth rates and stresses on metal absorption and (b) the root systems of both plants, because of their close proximity, should share the same soil base. Several sets of two such plants were selected solely for metal translocation studies; the soil directly around the plants was dislodged with a shovel to entirely include the complete root systems of both plants within the single soil sample base. This two-plant soil base combination then was placed in an upright position in a large plastic bag for shipment and later storage at −18 °C.

Treatment of Corn Plants and Soil. For analytical work, one set of two plants designated A and B was selected to represent unamended strip-mined soil. These two plants shared a soil base of 8.4 kg, of which 3.9 kg of the soil was adhering directly to both root systems. Counterpart plants designated C and D for the sludge-amended soil were larger and were associated with a soil base of 20.2 kg, of which 11.8 kg of the soil was adhering directly to the two root systems. Relative sizes of corn plants were in the order: C > D > A > B. Larger adhering soil samples reflected larger root systems and plants. The apparent root zone of the sludge-grown plants extended through a depth of 0–38 cm, whereas the unamended plant root zone depth was only 0–20 cm for these very small plants.

All soil not adhering directly to the root system was removed and discarded. All remaining soil attached directly to the root system was carefully removed by hand and air-dried in a hood. The dried soil sample was broken up and mixed on a large plastic sheet and then successively quartered and mixed until about 500 g remained. This sample was thoroughly mixed, and 125 g was ground with a mortar and pestle to pass through 100 mesh for subsequent chemical analysis. Both unamended and sludge-treated soils contained residual coal particles and had pH values of 7.4 and 7.5, respectively.

The ears of corn were weighed, husks were removed, and the kernels (moisture content 18.4 to 21.8%) were counted and weighed. Sections of the stems were broken and allowed to air-dry. The roots were immersed repeatedly in distilled water until the adhering soil was removed completely. All other tissues were rinsed with distilled water, air-dried, and placed in separate plastic bags.

ANALYTICAL TECHNIQUES

Flame atomic absorption was used to establish the metal content of soils, plant tissues, and soil leachates. Background correction with a hydrogen continuum lamp was used for lead and cadmium analyses. Comparative analyses were made with two Varian (Palo Alto, CA) spectrophotometers, Models 120 and AA6DAB. With both instruments only minor nonatomic absorption interference, if any, was demonstrated in the prepared aqueous sample solutions when analyzed by flame techniques.

Corn Tissues. Plant tissues were wet-ashed by two different procedures (Garcia et al., 1974a). Kernels were ashed with HNO_3 and $HClO_4$. The other more fibrous tissues, with higher levels of inherent silica, required an additional treatment with 48% HF for silica removal before treatment with $HClO_4$. Removal of silica eliminates metal adsorption on the residual siliceous material normally left after ashing, and it also prevents difficulties with these particulates in the final solution used for the flame analysis. For both ashing procedures, the final resultant solution (10 g of original sample/25 mL of 4% HCl) was the same.

Plant tissues were analyzed for mercury as previously described (Garcia et al., 1974b). Concurrent moisture determinations were established for alternate sections of the same tissue samples analyzed for metals.

Soils. It was intended to determine the total content of the seven metals in the soil. Decomposition of the soil (3 g) was accomplished by wet-ashing with nitric acid and removal of silica with 48% HF as previously described (Garcia et al., 1974a). The final resultant solution (250 mL of 10% HCl) was used directly for flame atomic absorption measurements.

For mercury analysis, soil (2 g) in a covered Phillips beaker was treated with 20 mL of an aqua regia mixture, containing concentrated HNO_3 and HCl (1:1) and heated at 70 °C for a period of at least 24 h until mercury salts were dissolved. For removal of oxides of nitrogen, 25 mL of double-distilled water was added, and heating was continued at the same temperature for an additional 8 h. The mercury salts in the diluted aqua regia solution were reduced chemically to the elemental form with stannous chloride. The mercury vapor produced was swept from the solution and measured by a nonflame atomic absorption technique.

Organic matter content was determined by oxidation with 0.1 N $K_2Cr_2O_3$, followed by iodometric measurement of the excess reagent. The unamended soil (3.8% organic matter) was slightly darker than the sludge-treated soil (1.6% organic matter) because of the presence of more ground coal particles. Residual coal is a carbonaceous material that will give higher values for organic matter content.

Table I. Mean Physical Characteristics of Corn Plant Components

characteristic	unamended plants A and B	sludge-grown plants C and D
wt of ear + husk, g	87.8	367
wt of ear, g	80.3	328
wt of cob, g	18.3	100
wt of kernels per ear, g	62.0	228
no. of kernels per ear	250	609
wt per 100 kernels, g	24.5	37.4

Soil Leachate Studies. Metal extractability studies were conducted to indicate metal availability to the corn plants. Soil samples (3 g) were extracted with 0.1 N HCl both at 70 °C and at ambient temperatures (20–24 °C). The hot extraction was intended to simulate longer contact periods between the corn plants and the soil matrix. The quantity of metal extracted by the treatment was compared to the total metal content previously established for the soil.

An all-glass system was used for the mercury extractability study. Immediately after the 0.1 N HCl extraction solution was made to volume (250 mL), three 80-mL aliquots of the solution were transferred to three bottles containing 10 mL of an aqua regia solution (HNO_3 and HCl, 1:1). The mercury then was determined as referenced previously.

RESULTS AND DISCUSSION

Corn Plant Characteristics. For both unamended and sludge-grown treatments, large and small plants were selected to establish whether the metals were translocated similarly within the same treatment even though the plants had not attained their full growth potential. Data in Table I for corn ear components corroborate data reported earlier (Garcia et al., 1974a) that showed that the sludge treatment increased the corn yield approximately fourfold. Although plant size differed considerably within each treatment, it was observed that kernel weight and quality were similar within each treatment. Kernels from the unamended soil were small and immature, reddish to yellow in color, with no characteristic indentation; whereas sludge-grown conditions produced larger well-developed yellow dented kernels.

Metal Content of Soils. Ideally, the actual soil medium from where the roots absorbed the metals is the most appropriate soil sample for chemical analysis to assess the transfer of individual metals from the soil to the plant. Furthermore, it was realized that soil samples formerly described (Garcia et al., 1974a) and collected within the same plots but at depths of 0–15 cm would give only an approximation of the soil metal content that would be associated with a particular root system. This was especially true in this case, where the sludge had been applied as a liquid topically to the soil.

In Table II, levels of heavy metals in soil samples in the plots at depths of 0–15 cm are compared to levels of the same metals occurring in the soil adhering to the root system. The results demonstrate the similarity of the unamended strip-mined soil with respect to metals content, even to the root depth of 0–20 cm. However, in the sludge-treated soil, plot samples formerly described from 0 to 15 cm do not reflect the metal content of the soil actually adhering to the plants at a root depth of 0–38 cm. These results show that the sludge-grown corn plant roots were actually associated with soil metal concentrations considerably lower than we formerly believed (by factors ranging from 1.32 for manganese to 5.05 for cadmium).

Table II. Heavy Metal Contents of Strip-Mine Soils and Metal Recoveries as Leached Out by Cold and Hot Extraction Treatments with 0.1 N HCl

	unamended soil					
	0–15 cm depth[a]			0–20 cm depth (root rhizosphere)[b]		
	total metal content,[c]	metal extracted with 0.1 N HCl, ppm		total metal content,	metal extracted with 0.1 N HCl, ppm	
element	ppm	cold	hot	ppm	cold	hot
zinc	113	8.0	27.7	119	12.7	30.3
manganese	759	336	424	737	424	460
copper	35.1	7.7	13.7	39.9	9.6	13.1
lead	28.7	6.7	12.5	32.8	9.9	16.5
chromium	81.2	0.6	5.4	81.3	1.1	4.4
cadmium	1.82	0.30	0.36	1.93	0.30	0.41
mercury	0.057	0.016	0.019	0.055	0.015	0.014
	sludge amended soil					
	0–15 cm depth[a]			0–38 cm depth (root rhizosphere)[b]		
	total metal content,[c]	metal extracted with 0.1 N HCl, ppm		total metal content,	metal extracted with 0.1 N HCl, ppm	
element	ppm	cold	hot	ppm	cold	hot
zinc	1107	672	701	257	144	166
manganese	772	403	468	583	296	359
copper	361	188	206	81.8	46.9	54.6
lead	235	138	145	64.4	37.9	44.6
chromium	574	122	198	162	19.9	44.4
cadmium	59.6	41.2	43.6	11.8	9.7	10.4
mercury	0.674	0.024	0.026	0.221	0.178	0.009

[a] Total metal content and metal extracted values listed for 0–15 cm depth levels, where no roots were present, represent the mean of three different soil samples collected within that plot in a triangular conformation approximately 15 m apart. [b] For both root rhizosphere levels, each value listed represents the mean of three individual replicates taken from the soil sample adhering to the root system. [c] Mean values of data formerly reported (Garcia et al., 1974a).

The manganese content in the sludge-treated soil was not expected to change much, since the added sludge contained minor quantities of manganese compared to quantities already present in the soil.

Results of Metal Availability Studies. No solvent or complexing agent in soil extraction studies is expected to give valid results with innumerable types of soils and at different soil pH ranges for a variety of metals. For these strip-mined soils, we selected 0.1 N HCl as the solvent.

From metal leaching data in Table II, the following conclusions were drawn: (a) Zinc, copper, lead, chromium, and especially cadmium were more available from sludge-amended soils. (b) Manganese was about equally available in both unamended and sludge-amended conditions. (c) Mercury in the unamended soils responded similarly with both cold and hot 0.1 N HCl extractions; however, in the sludge-amended soil at 0–15 cm the presence of considerably more organic material supplied by the topically applied sludge provided a reducing medium to reduce the mercury chemically. The elemental mercury thus produced probably volatilized. At the lower root level (0–38 cm depth), where the soil sample contained less sludge material, mercury was also volatilized but only with the hot 0.1 N HCl treatment. In the soil extraction study, only 0.1 N HCl was used initially and mercury losses could occur in a reducing medium. However, in the mercury analysis procedure, the initial addition of HNO_3 or aqua regia precludes any losses of mercury by volatilization, even during heating periods. To confirm the mercury volatilization the test was repeated four times with results almost identical with those shown on Table II. (d) Except for mercury, hot treatments of soils with 0.1 N HCl indicated an apparent higher metal availability.

Metal Accumulation in Tissues. The kernel is the tissue of most interest because it serves both as a source for a wide variety of human food products and as an important feed component; however, the other corn tissues are used as forage. Therefore, it is important to determine what tissues are more acceptable for feeding purposes as a result of differential metal accumulation for the tissues.

Plant uptake and distribution of the seven metals in the major components of the corn plant are shown in Table III with results given for each plant. Smaller plant size probably results from being grown under stress conditions where nutrients are not available to the plant.

The highest metal concentrations were found (1) in the roots for copper and chromium, (2) in the leaves for manganese, and (3) in roots and leaves for mercury, under both unamended and sludge-grown conditions. Highest concentrations of lead were found in the leaves with the unamended soil and in both roots and leaves after sludge application.

The effect of sludge application on tissue metal concentrations showed that: (a) Zn increased dramatically in roots and to a lesser extent in leaves. Zn distribution patterns were altered extensively thus resulting in higher concentrations in roots but lower concentrations in kernels. Plant size was also a factor in Zn accumulation in specific tissues. (b) Cd increased in roots, lower stems, and leaves at a considerably higher rate than for other elements. For these three tissues, concentrations of Cd were respectively 59, 7.55, and 5.52 times greater than in counterpart tissues representing untreated conditions. (c) Cu increased in roots, leaves, and stems; however, concentrations in husks, cobs, and kernels were similar for both untreated and sludge-grown plants. (d) Pb increased in roots, cobs, and possibly stems. Concentrations in other tissues were similar, except Pb content of kernels in untreated plants was higher. (e) Cr increased in roots, leaves, and possibly cobs and kernels. (f) As might be expected, concentrations of Mn were very similar for both untreated and sludge-grown plants because the quantity of Mn introduced to the soil by the sludge (approximately 4.5 μg of Mn/mL) was minor compared to quantities already present in the soil. (g) No discernible enhancement of Hg could be ascribed, except possibly in the kernels.

The effect of different availabilities of metals in the two different soils can be seen in concentrations of zinc and cadmium in tissues. These two metals were distributed similarly in the corn plant, and the content of these two metals was most altered as a direct result of the sludge application. For example, corn tissue from unamended soil contained lower relative concentrations of both Zn and Cd in roots and leaves, and the highest concentrations occurred in the upper stems of the smallest plant (plant B). In addition, concentrations of zinc and cadmium in the kernels were equal to or higher than in the root tissues. However, with the addition of sludge and a simultaneous higher metal availability, the highest metal concentrations again were in the roots and leaves; relative levels of these metals in the kernel were lowered.

Strip-mined soil usually contains coal and is considerably different from other soils and viable organic matter, containing rhizosphere organisms, is lacking and sometimes the quantity and the availability of trace elements, especially at soil pH's near 7, are lower. Consequently, corn plants grow under stress conditions and resultant kernels are considerably smaller.

After the soil was amended with sludge, nutrients increased, as well as both the quantity and availability of metals in the amended soil. Generally, the highest metal concentrations were found in the root and leaf tissues. Especially where metal availability is very high, such as for cadmium, root and leaf concentrations of the metal can be excessively high: however, even under these circumstances the plant still excludes the metal from the kernel and cob tissues.

In other studies where oats were grown in cadmium-contaminated soils, the roots contained high amounts of cadmium with smaller amounts in the above-ground portions (John et al., 1972a); in growth chamber studies, plant cadmium levels in radish and lettuce tops were significantly related to cadmium extracted with ammonium acetate from soils to which cadmium had been added (John et al., 1972b). Corn grown on a long-term sludge disposal site also contained higher amounts of trace elements in the roots (Kirkham, 1975).

To give a perspective of the uptake and translocation of metals to the corn plant for both unamended and sludge-amended strip-mined soils, a graphical combination (Figure 1) was made of metal concentrations occurring in (a) the soil adhering directly to the root system, (b) extractions of the same soil by hot and cold treatments with 0.1 N HCl, and (c) roots and leaves, where plant metal accumulation can best be demonstrated. The figure thus relates the total metal content of the soil medium to quantities of that metal that might be expected to be available to the plant, as determined by chemical means; this in turn is further related to metal concentrations (average of two samples) for two of the plant tissues.

SUMMARY AND CONCLUSIONS

In this study, the calcareous aspect of the strip-mine soil and the surface application of the sludge were two important considerations in the uptake of the metals. It would have been desirable to have had a larger number

Table III. Heavy Metal Concentrations (ppm, Dry Basis) for Corn Plant Tissues

corn plant component	unamended		sludge-grown		sludge-grown: unamended
	plant A	plant B	plant C	plant D	
			Zinc		
roots	23.2	23.9	71.0	74.6	3.09
leaves	61.5	44.8	84.8	72.4	1.48
upper stem	31.8	101.7	26.6	95.3	0.91
lower stem	40.3	93.3	24.3	43.7	0.51
husks	32.2	43.2	48.7	34.5	1.10
cob	31.7	76.3	38.1	33.9	0.67
kernels	34.1 ± 1.1[a]	34.6 ± 7.0	30.4 ± 0.4	27.2 ± 0.7	0.84
	(33.7)[b]	(18.7-27.2)[c]		(26.9)	
			Manganese		
roots	75.4	73.1	67.2	88.9	1.05
leaves	115.3	108.6	101.5	130.6	1.04
upper stem	17.2	20.0	15.3	37.6	1.42
lower stem	15.0	8.6	14.8	20.2	1.48
husks	35.9	57.6	37.5	41.6	0.85
cob	6.47	8.64	5.11	5.30	0.69
kernels	4.92 ± 0.19	5.15 ± 0.79	6.72 ± 0.07	5.91 ± 0.22	1.25
	(5.13)	(4.52-7.78)		(4.96)	
			Copper		
roots	14.8	13.9	39.6	52.0	3.19
leaves	4.00	4.54	13.1	10.3	2.74
upper stem	7.05	7.36	11.0	15.4	1.83
lower stem	5.64	3.78	15.1	13.0	2.98
husks	4.01	6.47	5.26	6.82	1.15
cob	3.22	2.89	3.46	2.81	1.03
kernels	2.53 ± 0.14	3.28 ± 0.73	2.05 ± 0.24	1.81 ± 0.22	0.66
	(2.55)	(1.65-3.10)		(2.49)	
			Lead		
roots	2.38	1.72	5.96	4.56	2.57
leaves	4.92	7.42	4.21	5.10	0.75
upper stem	0.773	0.880	0.510	2.24	1.66
lower stem	1.09	1.13	1.28	0.843	0.96
husks	4.24	3.31	1.80	2.81	0.61
cob	0.203	0.141	0.715	0.523	3.60
kernels	0.283 ± 0.019	0.330 ± 0.048	0.198 ± 0.124	0.145 ± 0.033	0.56
	(0.338)	(0.198-0.340)		(0.271)	
			Chromium		
roots	8.32	8.37	24.7	20.0	2.68
leaves	1.95	3.55	4.69	4.15	1.61
upper stem	0.328	1.94	0.197	2.01	0.97
lower stem	0.305	0.571	0.464	0.249	0.81
husks	1.05	2.91	0.688	0.573	0.32
cob	0.094	0.143	0.181	0.111	1.23
kernels	0.089 ± 0.011	0.086 ± 0.012	0.104 ± 0.033	0.113 ± 0.015	1.24
	(0.037)	(0.025-0.155)		(0.044)	
			Cadmium		
roots	0.073	0.050	3.71	3.55	59.02
leaves	0.355	0.196	1.42	1.62	5.52
upper stem	0.401	1.04	0.161	0.764	0.64
lower stem	0.030	0.024	0.208	0.200	7.55
husks	0.209	0.379	0.447	0.354	1.36
cob	0.066	0.080	0.055	0.145	1.37
kernels	0.071 ± 0.050	0.089 ± 0.073	0.052 ± 0.023	0.019 ± 0.002	0.44
	(0.033)	(0.035-0.148)		(0.067)	
			Mercury		
roots	0.0801	0.0631	0.0694	0.0836	1.07
leaves	0.0587	0.138	0.0748	0.0602	0.69
upper stem	0.0154	0.264	0.0112	0.0062	0.06
lower stem	0.0210	0.0068	0.0267	0.0136	1.44
husks	0.0262	0.0613	0.0212	0.0455	0.76
cob	0.0047	0.0292	0.0209	0.0248	1.34
kernels	0.0001 ± 0.0002	0.0017 ± 0.0011	0.0032 ± 0.0023	0.0025 ± 0.0021	3.16
	(0.00025)	(0.0018-0.0062)		(0.00075)	

[a] Results for the seven metals represent the mean and standard deviation for three individual whole kernel corn replicates (approximately 10 g each) from one cob. [b] In parentheses are shown the mean values (ppm) for six and five individual whole kernel samples withdrawn from other formerly reported (Garcia et al., 1974a) composite grain samples representing 56 and 20 ears of corn collected from the same plots for unamended and sludge-grown conditions, respectively. [c] Range of values for 11 different whole kernel samples grown under normal agronomic conditions, six of which originated from a six-state growth area (Garcia et al., 1974b).

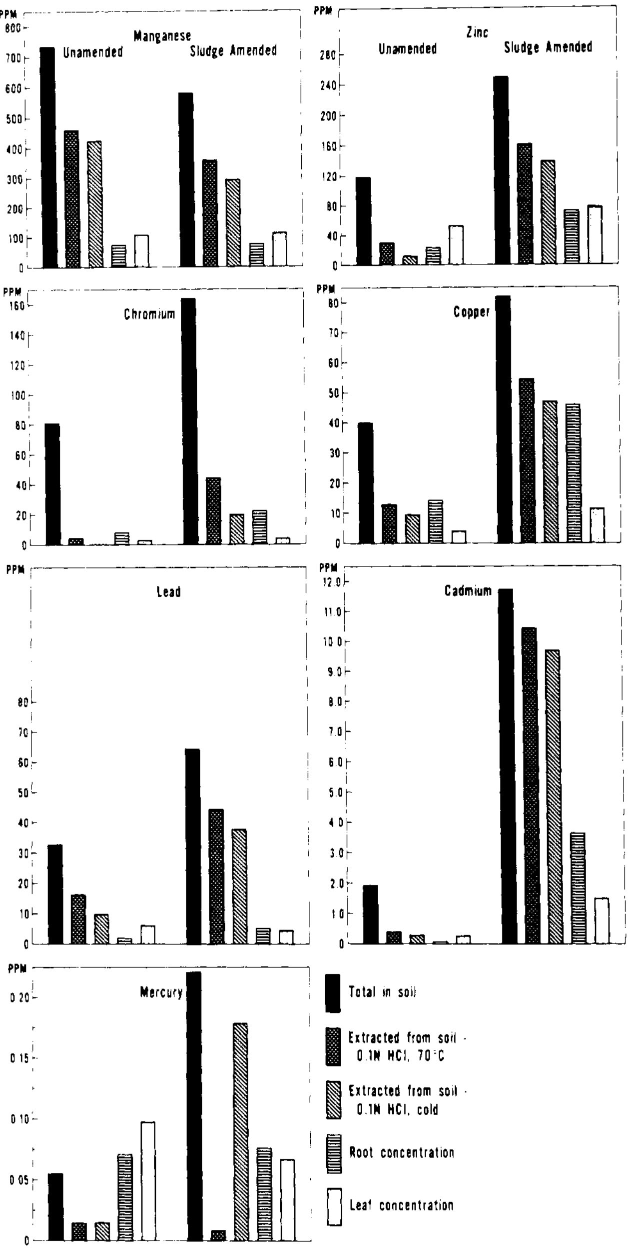

Figure 1. Graphical perspective of the translocation of seven metals from both unamended and sludge-amended soil to corn root and leaf tissues.

of the corn plants involved in the study; however, because the translocation of seven metals was studied, this was restrictive. A larger scale experiment would have added more definitive information.

Excessive quantities of Zn, Mn, Cu, Pb, Cr, Cd, and Hg were excluded by the plant from the kernels for conditions representing both untreated and sludge-grown plants. Thus no hazard to animals or humans would be anticipated when these grains enter the food chain. In addition, the cob, husk, and upper and lower stems, while generally having higher metal concentrations than the kernel, also show no excessive accumulation of metals and could be fed to animals. However, leaves and especially roots contained elevated metal concentrations as a direct result of the sludge application. The sludge-amended soil stimulated plant growth and higher concentrations of available metals were present, thus resulting in a metal accumulation pattern where the highest metal concentrations occurred in the root tissue. It also appears that as the metals concentrate in roots their translocation to other tissues is restricted by the plant. The sludge-borne phosphorus probably also had a similar effect in restricting the upward plant movement of metals. The roots accumulated concentrations of copper, lead, chromium, and cadmium, respectively, 3.19, 2.57, 2.68, and 59.02 times greater than counterpart roots grown under untreated conditions. Likewise sludge application increased: (a) copper accumulation in leaves (2.74 times) and in lower stems (2.98 times) and (b) cadmium in leaves (5.52 times) and lower stems (7.55 times).

Concentrations of copper and cadmium could become excessive in the root tissue, where the added sludge contains high concentrations of these two elements and where the availability of the metal in the amended soil matrix was demonstrated to be high. In addition, soils with a lower pH than in this study (7.4) would also make other metals such as lead, mercury, nickel, and manganese more available to the plant.

The element of greatest concern to the food chain is cadmium. The increased availability of cadmium to the corn plant, and its subsequent incorporation into specific plant tissues, is shown most dramatically in this study. Cadmium could pose a special problem to animal feeding where the total corn plant is used; however, consumption by animals and humans of the grain as grown under the conditions described here would constitute no hazard. Nordberg (1974) has summarized in detail the fate and known effects of cadmium in human beings and presents epidemiological data in problem areas of Japan.

A further consideration, especially as it affects the food chain, is the concept of the competitive nature between cadmium and zinc. Cadmium and zinc, which occur together in nature, have similar chemical characteristics (Cotton and Wilkinson, 1966), are closely associated in the soil and sludge, and thus compete for absorption by plants and animals. Biological benefits are derived by the assimilation of zinc (National Research Council, 1974), whereas deleterious toxic effects can result from cadmium incorporation. It has been postulated that a favorable percentage ratio of cadmium to zinc of less than 1.0% (Chaney, 1973) is most desirable. Ratios in the plant tissues that we studied give further evidence that the roots of the sludge-grown plants should be excluded from animal feed. Normal harvesting methods already do this.

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LITERATURE CITED

Boesch, M. J., *Compost Sci.*, **15**(1), 24 (1974).
Chaney, R. L., "Proceedings of the Joint Conference on Recycling Municipal Sludges and Effluents on Land", National Association of State University and Land-Grant Colleges, Washington, DC, 1973, pp 129–141.
Cotton, F. A., Wilkinson, G., "Advanced Inorganic Chemistry", Interscience, New York, 1966, pp 600–611.
Garcia, W. J., Blessin, C. W., Inglett, G. E., Carlson, R. O., *J. Agric. Food Chem.* **22**, 810 (1974a).
Garcia, W. J., Blessin, C. W., Inglett, G. E., *Cereal Chem.* **51**, 788 (1974b).
Hinesly, T. D., Braids, O. C., Dick, R. I., Jones, R. L., Molina, J. A. E., "Agricultural Benefits and Environmental Changes Resulting from the Use of Digested Sludge on Field Crops, Rept. No. DO1-UI-00080, U.S. EPA, Cincinnati, OH, 1972.
Hyde, H. C., *J. Water Pollut. Control Fed.* **48**, 77 (1976).
John, M. K., Chuah, H. H., Van Laerhoven, C. J., *Environ. Sci. Technol.* **6**, 555 (1972a).

John, M. K., Van Laerhoven, C. J., Chuah, H. H., *Environ. Sci. Technol.* 6, 1005 (1972b).
Kirkham, M. B., *Environ. Sci. Technol.* 9, 765 (1975).
Leeper, G. W., Reactions of Heavy Metals with Soils with Special Regard to Their Application in Sewage Wastes, Department of the Army Corps of Engineers under Contract No. DACW 73-73-C-0026, 1972.
National Research Council Recommended Dietary Allowances, 8th ed, National Academy of Science, Washington, DC, 1974, p 99.
Nicholas, D. J. D., in "Ecology of Soil Borne Plant Pathogens", Baker, K. F., Snyder, W. C., Eds., University of California Press, Berkeley, CA, 1965, p 210.
Nordberg, G., *Ambio* 3, 55 (1974).
Page, A. L., "Fate and Effects of Trace Elements in Sewage Sludge When Applied to Agricultural Lands", EPA-670/2-74-005, U.S. EPA, Cincinnati, OH, 1974.
Stover, R. C., Sommers, L. E., Silviera, D. J., *J. Water Pollut. Control Fed.* 48, 2165 (1976).

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Preparation and Nutritional Properties of Caseins Covalently Modified with Sugars. Reductive Alkylation of Lysines with Glucose, Fructose, or Lactose

Honson S. Lee, Lourminia C. Sen, Andrew J. Clifford, John R. Whitaker, and Robert E. Feeney*

Reducing sugars were attached to the ϵ-amino lysyl residues of casein in the presence of sodium cyanoborohydride, forming stable amine linkages. At pH 8.0 and 37 °C for 120 h in the presence of 1000 M excess of sugar, the degree of modification was 80% with glucose, 62% with fructose, and 17% with lactose. Compared to native casein, these reductively formed sugar derivatives of casein were shown to have lower in vitro digestibility by α-chymotrypsin and lower nutritive values in rat feeding experiments. Severe growth depression was observed in rats fed with the glucose-derivatized casein. Addition of lysine to the diet containing glucose-derivatized casein (71% modified) partially alleviated the growth-depressing effects. Moderate growth depression was observed in rats fed with the fructose-derivatized casein or with the lactose-derivatized casein. Since these three derivatives were not equally modified, the observed rat growth responses may be due to the decreased availability of modified lysine as well as to the nature of the attached sugar.

In developing new low-cost, high-quality protein foods such as beverages and bars (Altschul, 1969), proteins need modifying in order to obtain products with desirable characteristics (Feeney, 1977). Properties which can be affected by chemical modification include solubility at different pH values, susceptibility to heat denaturation, stability during storage, and extent of hydration and gel formation.

Evans et al. (1971) found that the polymer content of acyl β-casein derivatives increased with increasing *n*-alkyl chain length, suggesting aggregation via hydrophobic bonding. The *n*-hexanoyl, *n*-octanoyl, and *n*-decanoyl derivatives associated more strongly with increasing concentration and remained aggregated after dilution or equilibration at low temperature. Acetyl and propionyl β-caseins formed monomer–polymer systems similar to native β-casein, but they associated less strongly than β-casein due to their enhanced negative charge. Studies of the functional properties of acetylated and succinylated proteins in model systems have revealed improved heat stability, dispersion, foaming, and emulsification capacity (Groninger, 1973). Lee et al. (1978) have shown that methylation of as many as half of the side chain amino groups of lysines in casein does not lower the availability of lysine when the modified casein is fed to rats. Demethylation of methylated lysines was seen in the blood.

Attachment of hydrophilic groups (e.g., carbohydrates, phosphates) to proteins should also change their solubility, viscosity, hydration, and gel-forming characteristics. Although chemical phosphorylation is used mainly in the synthesis of nucleotides (Pettit, 1972), phosphorylation of proteins by chemical means has not been investigated extensively due to the harsh reaction conditions commonly used.

A number of methods for the covalent attachment of carbohydrates to proteins have been developed (Gray, 1974; Marshall and Rabinowitz, 1975; Krantz et al., 1976). The products obtained were used in immunological as well as enzyme heat stability studies. Marshall and Rabinowitz (1975) prepared soluble enzyme–carbohydrate conjugates by coupling trypsin, α-amylase, and β-amylase to cyanogen bromide-activated dextran. The conjugates were more stable to heat than the respective native enzymes. Loss of trypsin activity by autolytic hydrolysis was also decreased by attachment of carbohydrate. Attachment of D-galactosides to proteins enhanced rabbit liver membrane binding by several orders of magnitude, while attachment of D-glucosides enhanced binding to a variable extent depending on the method of linkage. Coupling of thioglycosides to proteins has been achieved by amidination, diazo coupling, and amide formation (Krantz et al., 1976).

Gray (1974) utilized a modification of the reductive alkylation reaction described by Means and Feeney (1968) for the direct coupling of reducing oligosaccharides to proteins with sodium cyanoborohydride in aqueous solution at pH 7. This procedure relies on the ability of the cyanoborohydride anion to selectively reduce the Schiff base formed between the carbonyl group of reducing sugars and the free amino groups of proteins. The secondary amine linkage thus formed is stable to acid hydrolysis. This reduction by sodium cyanoborohydride was shown

Department of Food Science and Technology and the Department of Nutrition, University of California, Davis, California 95616.

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