Cadmium and Zinc in Growing Sheep Fed Silage Corn Grown on Municipal Sludge Amended Soil

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Growing sheep were fed silage corn grown on soil amended with 280 dry metric tons per hectare of municipal sewage sludge from Syracuse, New York, for 274 days. Cadmium and zinc were found higher in specific organs of the animals fed sludge-grown corn silage. No significant differences were observed in hepatic microsomal mixed function oxidase activity or proliferation of hepatic smooth endoplasmic reticulum among the two treatment groups but histopathologic changes in hepatic sections of the sheep fed sludge-grown corn silage were observed by electron microscopy.

Millions of tons of municipal sewage sludge is generated annually in the United States. Disposal of this material has included ocean dumping or transporting of the treated sludge or incinerated sludge ash to sanitary landfills among others. A small percentage of municipal sludge has been sold as a dry bagged organic material in products such as Milorganite for use on lawns or ornamental plants.

Much research has been conducted recently on the feasibility of using sludge as a soil amendment for the growth of agronomic crops. Heavy metals such as Cd, Ni, Cu, Zn, and Pb as well as synthetic organic compounds like the polychlorinated biphenyls (PCBs) are typically present in municipal sludges (Furr et al., 1976a). Movement of metals from soils into plants growing on sludge-amended soils is well known and depends on many factors including soil type and pH, plant species, and rate of sludge application. The impact of metals in sludge on growing plants has been reviewed by Chaney (1973) and Page (1974). More recently, translocation of PCBs from soil (Iwata and Gunther, 1976) and sludge-amended soil (Lawrence and Tosine, 1977) to crops has also been reported.

Some research has been conducted on the transfer of potential toxicants from plants grown on sludge-amended soils to the tissues of foraging animals. Accumulation of cadmium and other elements in guinea pigs (Furr et al., 1976b; Chaney et al. 1978a; Babish et al., 1979), mice (Chaney et al., 1978b), voles (Williams et al., 1978), pheasants (Hinesly et al., 1976), swine (Hansen et al., 1976), and sheep (Haschek et al., 1979) fed crops grown on sludge-fertilized soils has been reported. PCBs have been found in the livers of guinea pigs (Babish et al., 1979) and sheep (Haschek et al., 1979) fed cabbage grown on municipal sludge. In this regard, enhanced hepatic microsomal mixed function oxidase activity (Hansen et al., 1976; Chaney et al., 1978b) and intestinal aryl hydrocarbon hydroxylase activity (Babish et al., 1979) indicative of ingestion of foreign organic compounds such as PCBs has also been shown in animals fed sludge-grown crops. A number of microscopic lesions in the liver and thyroid of sheep fed sludge-grown cabbage has been observed (Haschek et al., 1979). Also cattle consuming sludge-treated vegetation reportedly developed an arthritic or emaciated condition possibly caused by high iron intake and resultant iron deposits were found in their livers (Decker, 1978).

Much research has been conducted dealing with the growth of plants after municipal sludge has been applied to top soils. However, in many areas where topsoil has eroded or been mechanically removed, the resulting subsoil is typically much poorer in physical structure and fertility and might therefore benefit greatly from sludge incorporation. In the work reported, field corn grown on subsoil amended with municipal sewage sludge was ensiled and fed to sheep for 274 days. Analysis was made for elements in the sludge, soil and corn and for cadmium and zinc in animal tissues. Hepatic microsomal, mixed function oxidase activity was measured and histopathologic tissue examination using light and electron microscopy was also carried out.

EXPERIMENTAL SECTION

Sludge was obtained from the Ley Creek Sewage Treatment Plant in Syracuse, N.Y. Wastewater entering this facility is treated to produce an anaerobically digested, waste activated sludge. No lime or other chemicals are added during the treatment process. This plant receives the effluents discharged by about 100 industries as well as domestic wastes. The industrial activities represented include welding, plating, foundry, printing, laundering, fat rendering, and manufacture of bearings, die castings, gears, tools, steel and electrical products, china, paper board, chemicals, wood preservatives, beverage, dairy, and other food products.

In mid-April, 1977, 100 tons of the sludge (pH 6.5, 49.8% moisture) was trucked to Ithaca, NY, and applied to 0.4 acres of subsoil (approximately 280 dry metric tons/h). The sludge had been produced a year earlier during which time it had been allowed to weather to facilitate removal of excess soluble salts and decomposition of possible phytotoxic organic constituents. The sludge had a fertilizer equivalent of 0.7-1.0-1.4 (N-P-K) and contained 68.4% ash. The land used had consisted of top soil, Chenango gravelly loam (loamy-skeletal, mixed, mesic typic dystrochrepts), the upper 6 in. of which had been scraped off 12 years earlier. The exposed subsoil (pH 7.4) (46% sand, 43% silt, 9% clay, 2% organic matter; cation exchange capacity, 20 mequiv/100 g) had then layed in fallow until

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Table I. Ash, Fat, Protein, and Energy Content of Ration Constituents

constituent	ash, %	fat, %	protein, %	energy, cal/g
control corn silage	4.5	3.4	8.5	4526
sludge-grown corn silage	4.2	3.5	8.5	4509
soybean meal	6.5	1.4	54.0	4785

the time of sludge application. After spreading the sludge evenly with a bulldozer, the land was plowed, disced, and seeded with Cornell 407 hybrid field corn (*Zea mays*) (19300 plants/acre) at which time it was also fertilized with 350 lb/acre (393 kg/h) of 13.5–5.7–10.8 (N–P–K) ammophos fertilizer. The pH of the resulting sludge-amended soil was 7.0. The corn was side dressed with 150 lbs/acre (168 kg/h) of ammonium nitrate when the plants were about 12 in. in height. Similarly, 0.4 acres of the same subsoil was prepared, fertilized, and planted but without sludge addition to serve as the control. In October, 1977, the entire mature corn plants were field-chopped and placed in polyvinylchloride-lined silos (1.7 m diameter, 3 m high) in which they were sealed off from air for 3 months. The fresh weight yield of corn on the sludge-amended and control plots was, respectively, 14.4 and 12 tons/acre (32.8 and 27.3 metric tons/h).

Fourteen, 3-month-old Dorset wethers were used in the feeding trial. One part (dry weight) of soybean meal was mixed with 16 parts fresh weight of corn silage (75% moisture) to yield a total ration containing 17.6% dry weight of protein. Ash and fat were determined in the rations by the procedures cited, respectively, in the "Official Methods of Analysis" (AOAC, 1975). Protein was determined as Kjeldahl nitrogen × 6.25 and energy using a Paar bomb calorimeter. Table I lists the ash, fat, protein, and energy content of the rations.

Nine lambs were fed the sludge-grown corn silage ration and five animals were fed the control diet. The animals were located in individual metabolic stalls throughout the feeding period. They were adapted from a commercial pelleted lamb ration to the corn silage–soybean meal diet over a period of 10 days. They were then fed the latter ration for 274 days. Salt (without iodine or trace minerals) and water were provided ad libitum. Daily orts were weighed to allow calculation of actual total daily animal feed intake. The sheep were weighed monthly and at the end of the feeding period following an 18-h fast. They were then sacrificed by exsanguination. Samples of tissues, blood, and feces were taken for histopathologic examination, elemental analysis, and measurement of hepatic microsomal mixed function oxidase activity.

METHODS

Liver samples (0.5–1 mm in size) were fixed in 5% glutaraldehyde (50 mM sodium cacodylate, pH 7.5; 250 mM sucrose), followed by postfixation in 1% osmium tetroxide (same buffer as above). The liver pieces were washed several times in distilled water and then dehydrated through an ascending series of ethanol with two changes of propylene oxide and embedded in Epon-Araldite as described by Telford and Matsumura (1970). Thin sections were cut using a diamond knife mounted on a Sorval "Porter-Blum" MT-2 ultramicrotome. Sections were picked up on copper Athene type grids and poststained with uranyl acetate and Reynold's lead citrate (Reynolds, 1963). All examinations of the liver sections were done on an AEI EM6B electron microscope operated at an accelerating voltage of 80 kV. Sections of liver for light microscopic examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin.

Table II. Average (± Standard Error) Animal Weights, Feed Consumed, Rate of Weight Gain and Feed Efficiency

animal treatment ration[a]	initial body wt, kg	total feed consumed, kg	rate of wt gain, g/day	feed effic[b]
control corn silage	19.9 ± 1.8	520 ± 33	95.5 ± 4.0	0.05 ± 0.00
sludge-grown corn silage	20.9 ± 1.1	477 ± 14	78.7 ± 6.9	0.05 ± 0.01

[a] Differences between respective treatment means were not significant ($p > 0.05$). [b] Kilograms of animal weight gained/kilogram of ration consumed.

Table III. Total Elemental and PCB Content of Soil, Sludge, and Corn Grown Thereon

	concn (ppm, dry weight) in:			
element	soil	sludge	control corn	sludge-grown corn
Ag	1.9	31	1.7	4.1
Al	61760	34740	120	113
As	15	47	0.1	0.2
Au	0.01	0.4	0.01	0.01
B	5.2	9.0	6.6	5.5
Ba	476	293	25	22
Br	8.4	9.3	6.8	7.2
Ca	16600	106940	3436	4042
Cd	1.9	77	0.05	1.7
Ce	95	75	a	
Cl	1750	1100	4768	7468
Co	13	5.5	0.3	0.3
Cr	62	979	1.2	1.2
Cs	4.0	1.3	0.2	0.2
Cu	17	76	6.2	6.3
Dy	14	7.1	1.3	
Eu	1.2	1.2	0.1	0.02
Fe	31170	14150	177	166
Hf	8.9	5.5		
Hg	0.4	3.4	0.2	0.3
Ir	0.04	0.01	0.01	0.00
K	23600	14040	28160	28370
La	28	16	0.1	0.1
Lu	0.6	0.2	0.03	0.01
Mg	11870	11060	1779	1303
Mn	620	389	27	30
Mo	2.5	6.9	0.8	0.5
Na	5569	6855	20	17
Ni	27	145	0.4	0.5
Pb	22	384	2.5	2.8
Rb	113	51	2.7	8.2
Sb	1.7	6.6	0.8	0.5
Sc	7.2	2.1	0.01	0.01
Se	0.2	1.1	0.02	0.02
Sm	26	9.7	1.1	0.1
Ta	0.7	0.7	0.09	0.04
Th	27	12	1.4	0.3
Ti	5074	2291		
U	2.9	1.5		0.1
V	94	39	0.2	0.2
W	1.4	12	0.3	0.1
Yb	2.9	1.3	0.3	0.1
Zn	145	3477	19	68
PCBs[b]	0.07	8.5	0.04	0.03

[a] Absence of data was due to analytical interference. [b] Reported as Aroclor 1254.

Hepatic microsomal mixed function oxidase (MFO) activity in the animals was measured since this can be increased by various natural or synthetic organic compounds. Liver from each sheep was perfused in situ with a cold 0.9% NaCl solution, weighted, sliced, and homogenized in 4 volumes of ice cold 1.15% KCl containing 20

Table IV. Cadmium and Zinc (ppm, Dry Weight) in Sheep Tissues

tissue	cadmium in[a]		zinc in	
	control corn silage	sludge corn silage	control corn silage	sludge corn silage
bone	0.01 ± 0.001^{x}	0.02 ± 0.002^{y}	625 ± 58^{x}	662 ± 39^{x}
brain	0.01 ± 0.00^{x}	0.02 ± 0.00^{y}	53 ± 3^{x}	58 ± 2^{x}
heart	0.01 ± 0.00^{x}	0.03 ± 0.00^{y}	54 ± 7^{x}	59 ± 2^{x}
kidney	5.4 ± 0.8^{x}	18.5 ± 1.0^{y}	3271 ± 402^{x}	4135 ± 253^{x}
liver	1.2 ± 0.2^{x}	5.8 ± 0.3^{y}	1523 ± 261^{x}	1627 ± 161^{x}
muscle (chuck)	0.004 ± 0.001^{x}	0.01 ± 0.002^{y}	108 ± 3^{x}	151 ± 7^{y}
muscle (round)	0.005 ± 0.001^{x}	0.01 ± 0.001^{y}	95 ± 20^{x}	117 ± 5^{x}
spleen	0.04 ± 0.00^{x}	0.23 ± 0.02^{y}	102 ± 10^{x}	94 ± 3^{x}

[a] Mean ± standard error; dissimilar letter superscripts indicate significant differences ($p < 0.05$).

Table V. Average (± Standard Error) Liver Protein, Hepatic Microsomal Mixed Function Oxidase Activity, and Liver/Body Weight Ratios of Sheep Fed the Two Rations

animal treatment ration[a]	mg of protein/g of liver	aminopyrene *N*-demethylase, nmol (mg of protein)$^{-1}$ h^{-1}	*p*-nitroanisole *O*-demethylase, nmol (mg of protein)$^{-1}$ h^{-1}	liver/body wt, %
control corn silage	25.22 ± 0.25	5.94 ± 0.49	8.02 ± 0.41	1.27 ± 0.03
sludge-grown corn silage	24.18 ± 0.46	9.27 ± 1.59	10.07 ± 0.73	1.27 ± 0.04

[a] Differences between respective treatment means were not significant ($p > 0.05$).

mM Tris-HCl buffer, pH 7.4, using a Potter-Elvehjem Teflon–glass homogenizer fitted to a mechanical drill. Enzyme activity was estimated by measuring the product of formation of two incubated substrates with the 12000*g* supernatant liver fraction with an NADPH generating system (Conney, 1967). *p*-Nitroanisole *O*-demethylase activity was measured by determining the *p*-nitrophenol produced (Kato and Gillette, 1965) and aminopyrene *N*-demethylase activity was determined by measuring the production of formaldehyde (Nash, 1953). Microsomal plus soluble protein was analyzed by a modified Lowry procedure (Sutherland et al., 1949).

Comparison of means (Tables II, IV, and V) was accomplished by Student's *t* test as described in Steel and Torrie (1960). Nondestructive neutron activation analysis of the freeze-dried sludge, soil, and plant tissue for 36 elements was conducted by the procedure described earlier (Furr et al., 1976b). Boron was determined by the curcumin spectrophotometric procedure (Greweling, 1966). Cadmium, copper, lead, and zinc were determined by conventional stripping voltammetry using a Princeton Applied Research Corp. Model 174 polarographic analyzer by the procedure of Gajan and Larry (1972). Nickel was measured by furnace atomic absorption using a Perkin Elmer Model 303 spectrophotometer equipped with an HGA-2000 furnace and deuterium background corrector. Phosphorus was determined by the molybdivanadophosphoric acid spectrophotometric method (Greweling, 1966). The determination of selenium was performed by the fluorescence method of Olson (1969). The method of Peech et al. (1953) was used to measure pH. Soil, sludge, and corn plant material were analyzed for PCBs as Aroclor 1254 using electron-capture gas chromatography (Pesticide Analytical Manual, 1971).

RESULTS AND DISCUSSION

The averages for initial animal body weights, total feed consumed, rate of weight gain, and feed efficiency (kilograms of weight gain/kilogram of ration consumed) are given in Table II. It is noteworthy that the sheep fed the sludge-grown corn silage initially showed a marked reluctance to consume their ration. Only after several weeks did their silage intake begin to approach that of the control animals. Their average total intake for the entire experiment was therefore notably less than that of the control sheep (see Table II). It is possible that this was due to absorption by the corn of synthetic organic compounds other than PCBs (see Table II) from sludge which survived ensiling and altered the taste and acceptability of the ration. It is also possible that particles of sludge splashed onto the corn by rain prior to harvesting may have contributed off flavors in the diet.

The results of analysis of the soil, sludge, and corn for 43 elements and PCBs are listed in Table III. Silver, As, Au, B, Br, Ca, Cd, Cr, Cu, Hg, Mo, Na, Ni, Pb, Sb, W, and Zn were higher in concentration in the sludge than the soil. Of the elements of toxicologic interest Cd and Zn were appreciably higher in the sludge-grown corn as compared to the control crop. Cadmium absorption by the sludge-grown corn was appreciable even though the neutral pH of the sludge-soil mixture would reduce the availability of Cd to plants. Interestingly, Ag was also higher in the sludge-grown corn undoubtedly reflecting its presence in the sludge. PCBs were not detected at a higher level in the sludge-grown corn.

The average concentrations of Cd and Zn in various tissues of the sheep are given in Table IV. Cadmium expectedly concentrated in the kidney and liver (Browning, 1969) and to a lesser extent in spleen in the animals fed the sludge-grown corn silage. Zinc was higher in all organs except spleen of the sludge-corn fed animals, but only the higher mean zinc value for muscle was significantly different ($p < 0.05$) than that of the control animals. Metallothionein, a low-molecular-weight protein present in kidney and liver, is responsible for binding heavy metals such as Cd and Zn (Shaikh and Smith, 1976). Cadmium and zinc are also potent inducers of this protein (Magos and Webb, 1978). This protein has been identified in liver and kidney of cows, swine, and chickens (Verma et al., 1978).

Histologic examination of liver sections by light microscopy showed no differences between control sheep and those fed the sludge-grown corn silage. By electron microscopy greatly enlarged and swollen mitochondria and necrosis were seen in hepatocytes of each of the sheep fed the sludge-grown corn silage. Mitochondria were normal and necrosis was absent in hepatocytes of all of the control animals. The cause of these early signs of liver cell degeneration is unknown but swollen hepatic mitochondria (Faeder et al., 1977) and degenerating hepatocytes (Colucci et al., 1975) have been observed in rats exposed to cadmium. Cadmium has been shown to diminish the re-

spiratory control normally exerted by mitochondria (Diamond and Kench, 1974).

No significant differences were observed in liver protein, hepatic MFO activity or the liver to body weight ratio between the two animal teatment groups (Table V). This was also supported by the fact that no differences in proliferation of smooth endoplasmic reticulum were observed between the treatment groups. MFO activity typically reflects the effects of a variety of foreign synthetic and natural compounds ingested by animals. PCBs and polynuclear aromatic hydrocarbons are examples of compounds which are known to induce MFO activity when ingested by animals. Conversely, heavy metals such as cadmium can inhibit MFO activity (Becking, 1976). It is possible, therefore, that the absence of enhanced hepatic MFO activity in the sheep fed sludge-grown corn silage reflected a balance between MFO enhancement by synthetic organic compounds other than PCBs and inhibition by metals such as Cd.

In summary, this study indicates that corn for silage grown on sludge-amended soil at pH 7 absorbed an appreciable concentration of Cd. Corn varieties, however, differ greatly in their ability to absorb Cd from sludge-amended soils (Hinesly et al., 1978). Cadmium absorption would presumably also be enhanced by higher equivalent Cd-sludge applications or a more acid soil. Since the corn silage comprised the major portion of the animal diet in this study, absorption of cadmium by animal tissues would expectedly be enhanced. While feeding this corn did significantly increase the kidney and liver content of Cd in sheep it did not appreciably increase its concentration in muscle tissue which would be consumed by humans. This is important since Cd is toxic to humans (Brown et al., 1978) and although Zn counteracts Cd toxicity the deposition of Zn in humans is poor (Browning, 1969). Depending on the amount consumed, the use of significant amounts of Cd-containing sheep organs such as kidney and liver in pet foods might constitute a hazard not only to pets but to humans among poorer classes who may consume such products. Fortunately, however, the Zn content of such tissues may also be high and this may afford a protective effect.

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