Cadmium Deposition and Hepatic Microsomal Induction in Mice Fed Lettuce Grown on Municipal Sludge-Amended Soil

Romaine lettuce, cultured on soil amended with municipal sludges, was fed as 45% of their diet to mice. The concentrations of Cd and Zn in the lettuce correlated closely with the element levels in the respective sludges on which they were grown. When compared to control mice, Cd showed significantly ($p < 0.05$) higher concentration in the kidneys of mice fed Baltimore, Milwaukee, and a Washington, D.C. sludge-grown lettuce. The hepatic microsomal enzyme, *p*-nitroanisole *O*-demethylase was significantly ($p < 0.05$) higher than controls in the mice fed the lettuce grown on Milwaukee sludge.

Sewage sludges resulting from municipal wastewater treatment may typically contain a wide variety of elements resulting largely from industrial activities. A host of synthetic organic compounds may also be present depending on the spectrum of industries served. While sludge is currently disposed mainly by ocean dumping, incineration, and land filling, much research is underway to study the feasibility of its use as a fertilizer and soil conditioner in agriculture and horticulture. Elements such as Cd, Cu, Ni, Zn, and others commonly present in relatively high amounts in sludges (Furr et al., 1976a) may be absorbed by plants.

Chaney (1973) and Page (1974) have reviewed the effects of soil pH and other factors on the extent of uptake of such elements by plants. Toxic refractory organic compounds such as the polychlorinated biphenyls (PCBs) also common in sludges (Furr et al., 1976a) have been reported to be absorbed by plants from soil (Iwata and Gunther, 1976) or sludge-amended soils (Curry, 1977; Lawrence and Tosine, 1977). Relatively little research has been done to study the movement of toxicants in sludge-grown crops to tissues of foraging animals. Furr et al. (1976b) cultured Swiss chard on a silt loam soil which had been amended with 100 dry tons/acre of municipal sludge. When the chard was fed as 45% of their diet to guinea pigs for 28 days, markedly higher concentrations of Cd, Mn, Sn, and Sb were found in the animal tissues than in those of control animals. Owing to its known toxicity and mobility (Lisk, 1972) in passage from soil to plants and deposition in foraging animal tissues, cadmium is the element of most concern in municipal sludges. Zinc is also of interest since it is known to counteract the toxic effects of Cd in animals (Parizek, 1957).

Hansen et al. (1976) reported enhanced hepatic microsomal mixed-function oxidase activity in swine fed sewage sludge-fertilized corn. Stoewsand et al. (1977) observed an increased aryl hydrocarbon hydroxylase activity in guinea pigs fed cabbage grown on Syracuse, New York municipal sewage sludge. This enhanced microsomal enzyme activity is indicative of liver and intestinal challenge by foreign organic compounds and is considered a major mechanism of detoxication. More than 200 unrelated chemical compounds are known to stimulate hepatic microsomal enzyme activity (Conney, 1967). In this study, romaine lettuce was cultured in soil-amended with sludges from several cities. The lettuce was fed to mice as a portion of an otherwise complete diet, and sludges, plant, and animal tissues were analyzed for Cd and Zn. Hepatic microsomal enzyme induction was also determined in these mice.

EXPERIMENTAL SECTION

The plant studies were conducted on field plots at the Beltsville Agricultural Research Center, Beltsville, Md. The soil was a Woodstown silt loam. Its cation-exchange capacity was 5.9 mequiv/100 g. The treatments included (1) an anaerobically digested sludge from the Back River Water Pollution Control Plant in Baltimore, (2) an anaerobically digested sludge from the Blue Plains Water Pollution Control Plant in Washington, D.C., (3) the latter Blue Plains digested sludge composted with wood chips by the windrow method (Epstein and Wilson, 1975), (4) a commercially available, heat-dried, raw secondary sludge (Milorganite), and (5) a Blue Plains mixed raw sludge heat-dried in a torroidal flash drier and containing about 50% w/w Milorganite which was used in the start up of the drier (dried sludge A). Addition of wood chips to sludge (see no. 3 above) and windrowing is believed to promote aeration and result in more rapid oxidation of undesirable synthetic organic constituents which may inhibit plant growth. Table II lists these sludges, their rates of application to the soil, and their total Cd and Zn content.

The sludge materials were incorporated into the soil using rotary cultivation. Swiss chard was planted in the plots in 1974 (Chaney et al., 1978). Dolomitic limestone was also worked in to adjust the soil to pH 6.0. All plots were fertilized with potassium. Phosphorus and nitrogen fertilizer was used only on the control soil.

"Paris White" romaine lettuce (*Lactuca sativa*) was grown to maturity in 1975. At harvest, the lettuce leaves were washed by repeatedly dipping in 0.1% sodium lauryl sulfate solution for 15 s, followed by thoroughly rinsing with water. The crop material was freeze-dried, milled to a powder, and mixed.

Weanling, male, albino, CDF mice were caged in six groups of seven mice/group in suspended stainless steel wire cages with diets and water supplied ad libitum. Lettuce grown on the five sludges (and control) were each fed at 45% of an otherwise purified "American Institute of Nutrition–1976" diet (Bieri et al., 1977) being substituted for the carbohydrates and fiber. The dietary constituents are listed in Table I. Control lettuce was grown on soil alone. At the end of 6 weeks, four mice per each of the six treatments were sacrificed by decapitation following a 10-h fast. Livers were perfused in situ with a cold 0.9% NaCl solution, weighed, sliced, and homogenized

Table I. Composition of Mouse Diets

Constituent	Percent dry weight
Lettuce	45.0
Casein	20.0
DL-Methionine	0.3
Cornstarch	5.0
Sucrose	20.0
Corn oil	5.0
Mineral mix	3.5
Vitamin mix	1.0
Choline bitartrate	0.2

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in 4 volumes of ice-cold 1.15% KCl containing 20 mM Tris-HCl buffer, pH 7.4, using a Potter-Elvehjem Teflon-glass homogenizer fitted to a mechanical drill.

Hepatic microsomal enzyme activity was estimated by measuring product formation of two incubated substrates with the 12 000*g* supernatant liver fraction in 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 measurement of the production of formaldehyde (Nash, 1953). Microsomal protein was analyzed by a modified Lowry procedure (Sutherland et al., 1949).

Kidneys and liver were analyzed for Cd and Zn at the end of the 11-week feeding period. The plant and animal tissues were freeze-dried and dry ashed, and Cd and Zn were determined by conventional stripping voltammetry using a Princeton Model 174 polarographic analyzer (Gajan and Larry, 1972).

Comparison of means (Tables IV and V) was accomplished by Duncan's new multiple range test as described in Steel and Torrie (1960).

RESULTS AND DISCUSSION

In Table II listing the Cd and Zn contents of the sludges, the concentrations of these elements in the Blue Plains compost were much lower than in the corresponding Blue Plains sludge. It is possible that the effect of composting resulted in oxidative conversion of insoluble forms of the elements, e.g., metallothioproteins or sulfides, into more soluble forms such as chelates or sulfates which were subsequently leached out by rainfall. The concentrations of Cd and Zn in the lettuce grown on the various sludge treatments are given in Table III. The levels of Cd and Zn in the plant material correlates closely with those in the respective sludges. Absorption of Cd by the lettuce did not appear to be affected by the Zn content of the respective sludge. Fixation of Zn by the soil at pH 6.0 or selective absorption of Cd by the lettuce may have accounted for this. The concentrations of Cd and Zn found in liver and kidney of the mice fed lettuce from the various treatments are listed in Table IV. Cadmium showed significantly ($p < 0.05$) higher concentrations in the kidneys of mice fed Back River, Milorganite, and dried sludge A grown lettuce as compared to those fed control lettuce. Cadmium residues in kidneys and livers of mice fed Milorganite-grown lettuce was four and six times higher, respectively, than the control tissues. There was no significant increase in the concentration of zinc in animal tissues. Absorption of Zn by the gastrointestinal tract is reportedly variable in animals and poor in human beings (Browning, 1969). The form of Zn in the lettuce would affect its extent of deposition. Also the total content of Zn in the diet (Table I) may have exceeded the saturation level for deposition of the element in animal tissue above that observed in the control mice. In our study Cd concentration is reported on a whole kidney basis but the element is known to concentrate particularly in the kidney cortex. The deposition of Cd has been reported in tissues of pheasants fed corn (Hinesly et al., 1976) and guinea pigs fed Swiss chard (Furr, et al., 1976b) grown on sludge-amended soils. Dietary Zn, as present in plants with Cd, has been reported to diminish deposition of Cd in tissues of rats (Welch et al., 1978). This antagonism by Zn, if operative in this study, was not sufficient in magnitude to completely offset deposition of Cd in the tissues of the mice fed the Milorganite and dried sludge A grown lettuce since these latter crops were much higher in Cd than the control crop.

The mice from all treatments appeared healthy with similar body weight gains and normal tissues upon gross examination. Liver weights of the mice on the Milorganite treatment were (% of body weight) 4.67 ± 0.14 compared to a control of 4.35 ± 0.12. This was not a significant increase ($p > 0.05$) in liver weights. However, the hepatic microsomal enzyme, *p*-nitroanisole *O*-demethylase was significantly ($p < 0.05$) elevated above the control in the mice fed the Milorganite-grown lettuce (Table V). Most studies show that orally administered Cd alters hepatic microsomal metabolism by inhibition rather than stimulation (Becking, 1976) although the interrelationship of Zn could alter this effect (Becking and Morrison, 1970). It is more likely that organic compounds such as halo-

Table II. Soil Application Rates and Cd and Zn Content of Sludges

Sludge	Application rate, mt/ha[a]	Ppm, dry weight	
		Cd	Zn
Back River	56	15	5460
Blue Plains	112	20	2690
Blue Plains compost	224	6.7	930
Milorganite	56	90	1420
Dried sludge A	56	59	1290

[a] Metric tons/hectare.

Table III. Concentrations of Cd and Zn in Romaine Lettuce Grown on Soil Amended with the Various Sludges

Sludge treatment	Ppm, dry weight	
	Cd	Zn
Control (soil alone)	0.6	47
Back River	1.7	419
Blue Plains	2.5	307
Blue Plains compost	1.1	75
Milorganite	26.4	187
Dried sludge A	11.6	209

Table IV. Mean Residues of Cd and Zn in Kidney and Liver of Mice after 11 Weeks of Being Fed Lettuce Grown on Various Municipal Sludges

Sludge treatment	Ppm, dry weight			
	Liver[a]		Kidney[b]	
	Cd	Zn	Cd[c]	Zn
Control (soil alone)	4.1	109	10.8 ± 0.9^c	60 ± 9.5
Back River	7.6	92	17.5 ± 2.2^b	62 ± 1.2
Blue Plains	7.8	102	13.8 ± 1.0^c	64 ± 2.4
Blue Plains compost	2.9	91	6.9 ± 0.4^d	61 ± 1.5
Milorganite	25	99	48 ± 1.8^a	66 ± 1.5
Dried sludge A	5.5	92	17.8 ± 0.7	61 ± 2.6

[a] Tissues from two animals per treatment. [b] Tissues from four animals per treatment. [c] Mean ± standard deviation; common letter superscripts indicate nonsignificant differences ($p > 0.05$).

Table V. Effect of Sludge-Grown Lettuce on Mouse Liver Microsomal Enzyme Activity

Treatment	Enzyme activity[a]	
	p-Nitroanisole *O*-demethylase[b]	Aminopyrine *N*-demethylase
Control (soil alone)	10.98 ± 0.91^b	1.43 ± 0.23^{xy}
Back River sludge	10.88 ± 0.88^b	1.41 ± 0.21^{xy}
Blue Plains sludge	10.45 ± 0.44^b	1.26 ± 0.12^y
Blue Plains compost	11.21 ± 1.57^b	1.71 ± 0.25^{xy}
Milorganite	14.95 ± 0.73^a	1.92 ± 0.18^x
Dried sludge A	11.57 ± 0.69^b	1.68 ± 0.11^{xy}

[a] Expressed as nmol of product (mg of protein)$^{-1}$ hour.$^{-1}$ [b] Mean ± SE; common letter superscripts indicate nonsignificant differences ($p > 0.05$).

genated hydrocarbons, polycyclic aromatic hydrocarbons, etc. translocated from the sludge to the lettuce and ultimately to the mice caused this induction. When measuring enhanced microsomal enzyme activity by the rate of O-dealkylation of *p*-nitroanisole, either phenobarbital or polycyclic hydrocarbon types of compounds are about equally potent inducers. No observed stimulation of N-dealkylation using aminopyrene as a substrate indicates that a polycyclic hydrocarbon was involved (Conney, 1967).

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Elemental Content of Tissues of Guinea Pigs Fed Swiss Chard Grown on Municipal Sewage Sludge-Amended Soil

Swiss chard was grown on soil amended with municipal sewage sludges from Baltimore and Washington, D.C. The harvested crops were fed at 20 or 28% of the diet to guinea pigs for 80 days. Samples of soil, sludges, plant, and animal tissues were analyzed for up to 43 elements. The elements Br, Ca, Co, Eu, Fe, Ni, and Sr were found at higher concentrations in tissues of animals fed the chard cultured on sludge-amended soil than in control animals. Composting sludge prior to amending the soil appeared to render certain elements such as Cd, Cu, Ni, and Zn less available to Swiss chard subsequently grown.

Municipal sewage sludges are currently disposed by ocean dumping, trucking to landfills, or incineration. These methods may result in water or air pollution. Recently, investigators have turned their attention to the feasibility of land application of sludge as a fertilizer and soil conditioner for the growth of agronomic, vegetable, ornamental, or forest plants. Toxic metals in sludges such as Cd, Ni, Pb, Zn, and others originating largely from

