Elemental and Polychlorinated Biphenyl Content of Tissues and Intestinal Aryl Hydrocarbon Hydroxylase Activity of Guinea Pigs Fed Cabbage Grown on Municipal Sewage Sludge

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Guinea pigs were fed cabbage grown on municipal sewage sludge from Syracuse, NY, or soil (control) as 45% of their diet for 100 days. Among 45 elements determined, Cd, Cu, Ni, Pb, and Zn were higher in the sludge-grown cabbage and specific tissues of the respectively fed animals than controls. Cadmium was elevated in kidney, liver, and spleen, while only liver exhibited increased lead levels in the guinea pigs fed the sludge-grown plants. Polychlorinated biphenyls were found at higher concentrations in the sludge-grown cabbage and livers of the respectively fed guinea pigs than in the controls. Intestinal aryl hydrocarbon hydroxylase activity was 129% higher than controls in the animals fed the sludge-grown cabbage. All animals grew normally and showed no signs of overt heavy metal toxicosis.

Sewage sludge is the solid end product remaining after treatment of domestic and industrial wastes. Millions of tons of this material are produced annually in the United States containing a galaxy of organic and inorganic chemicals and microorganisms. Whereas much of this material has been disposed of by ocean dumping, incineration, and landfilling, appreciable numbers of individuals haul sludge from municipal treatment plants to make heavy applications on gardens for growing vegetables and other food crops since it is high in nitrogen and other plant nutrients.

Recent concern is being focused on the levels of certain toxic elements in municipal sewage sludge such as cadmium, lead, nickel, zinc, and others, as well as industrial organic compounds such as the polychlorinated biphenyls (PCBs) (Furr et al., 1976a) and to the magnitude of their absorption by edible crops (Page, 1974). Very little information is available on the movement of such toxicants from food crops to the consumer. In the work reported, cabbage grown on municipal sewage sludge or soil (control) was fed to guinea pigs as a portion of a complete diet for 100 days. Analysis of growth media and plant and animal tissues were made for 45 elements. The activity of intestinal aryl hydrocarbon hydroxylase (AHH) in the animals on both dietary treatments was also measured to learn if enzyme induction may have occurred as a result of ingesting foreign compounds that may have translocated into the sludge-grown cabbage.

EXPERIMENTAL SECTION

The plant growth portion of the study was conducted at the Ley Creek Treatment Plant in Syracuse, NY. This facility produces an anaerobically digested, waste activated sludge using no lime or other added chemicals during the treatment process. In addition to domestic wastes, this plant receives the effluents discharged by about 100 industries. 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, paperboard, chemicals, wood preservatives, beverage, dairy, and other food processes.

Table I. Guinea Pig Diets

ingredients	percent
cabbage (dried)	45.0
casein (vitamin-free)	28.0
sucrose	7.9
corn oil	7.5
minerals[a]	6.0
potassium acetate	2.5
magnesium oxide	0.5
choline chloride (50%)	0.4
ascorbic acid	0.2
vitamin mix	1.0
L-argenine	1.0

[a] See Reid and Briggs, 1953.

A site was chosen near the treatment plant on which sewage sludge, 4 ft in depth, had been placed following treatment 18 months earlier. During this period of time, rain would have leached excess salts out of the sludge and further microbiological decomposition of possible phytotoxic organic wastes (industrial cutting oils, for instance) would have occurred. An area 50 by 90 ft was leveled and 60 lb of 10-10-10 fertilizer was tilled into the upper 10 in. of sludge using a rotary cultivator. The pH of the sludge was 7.3 and it had a fertilizer equivalent of 0.55/0.41/0.024% N-P-K. In May, 1975, 500 transplants of "Green Winter" cabbage (*Brassica oleracea* var. *Capitata*) were planted in rows 36 in. apart with 24 in. between plants. Each plant was fertilized with 250 mL of a soluble 20-20-20 fertilizer solution. The plot was fenced, and the plants were weeded and cultivated weekly. In September the heads were harvested, care being taken to discard the outermost leaves which may have been contaminated with sludge debris. The heads were washed, shredded, and dried at 100 °F for 144 h and at 165 °F for an additional 48 h. The total yield of cabbage from the plot was 1900 lb fresh weight (120 lb dry weight). The dried material was then milled to a fine powder and thoroughly mixed. An equal number of heads of the same variety of cabbage was grown on Honeoye silt loam soil, pH 6.5, in Brockport,

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NY, to serve as controls. They were similarly harvested, dried, and processed. The dried plant material from each treatment was then incorporated 45% by weight into complete diets for guinea pigs. The dietary ingredients are listed in Table I.

Young, male albino guinea pigs of the Hartley strain with an average weight of 200 g were caged in pairs in suspended stainless steel wire screen units. Ten animals were fed each diet. The cabbage diets were gradually introduced to the animals, initially fed a pelleted commercial ration. After 4 days they were fed only the semipurified, dried cabbage diet (Table I) for 100 days. These diets contained all known required vitamins and minerals with the cabbage substituted for the common fiber and part of the carbohydrate ingredients described previously (Stoewsand et al., 1973). At the end of the feeding period the animals were sacrificed by a quick head blow, and their tissues were excised.

The activity of intestinal aryl hydrocarbon hydroxylase (AHH) in the animals was measured by the following procedures: A 20-cm segment of the small intestine, measured from the pyloric valve of the stomach, was removed and homogenized in 5 mL of ice-cold 1.15% KCl containing 20 mM Tris-HCl buffer, pH 7.4 (at 37 °C). The postmitochondrial supernatant used in assaying the intestinal AHH activity was obtained after two successive centrifugal steps, i.e., 2000*g* for 10 min and 12000*g* for 20 min. Decanting the post-mitochondrial supernatant glass wool prevented fat and cellular debris from passing.

The 2-mL incubation mixture contained 1 mL of the 12000*g* supernatant; 0.15 M KCl; 20 mM Tris-HCl buffer, pH 7.4 at 37 °C; 1.2 mM NADP; 9.8 mM isocitrate; 6.0 mM $MgCl_2$; 0.18 unit of isocitrate dehydrogenase; and 100 μM benzo[*a*]pyrene. This mixture was incubated under ambient air in 30-mL beakers using a Dubnoff shaking incubator at 37 °C. All observations were made during a time interval when reaction rates were linear. Reactions were initiated by addition of the substrate and terminated with 2 mL of ice-cold acetone.

With benzo[*a*]pyrene as the substrate, the aryl hydrocarbon hydroxylase system was assayed by determining the amount of 3-hydroxybenzo[*a*]pyrene formed. After terminating the reaction with the addition of acetone, 6.5 mL of hexane was added, and the mixture was incubated with shaking at 37 °C for 10 min. A 1.0-mL sample of the 6.5-mL organic phase was extracted with 3.0 mL of 1 N NaOH. The concentration of 3-hydroxybenzo[*a*]pyrene was determined fluorometrically with activation at 396 nm and fluorescence at 522 nm. All measurements of fluorescence were made within 5 min of alkali extraction since the 3-hydroxybenzo[*a*]pyrene is unstable at high pH. Activity of the AHH system was expressed as picamole of product per milligram of postmitochondrial protein per minute.

Nondestructive neutron activation analysis of freeze-dried sludge and plant and animal tissues for 35 elements was conducted by the procedures previously described (Furr et al., 1976a). Cadmium, lead, zinc, and copper 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 determined by furnace atomic absorption using a Perkin Elmer Model 303 spectrophotometer equipped with an HGA-2000 furnace. The determination of selenium was performed by a modification of the method of Olson (1969) employing wet digestion of the sample and measurement of the fluorescence of piazselenol resulting from reaction of selenium with 2,3-diaminonaphthalene. Arsenic was determined by dry ashing (Evans and Bandemer, 1954) the samples, distilling arsine, and analysis using the silver diethyldithiocarbamate spectrophotometric procedure (Fisher Scientific Co., 1960). Boron was determined by the curcumin spectrophotometric procedure (Greweling, 1966). Phosphorus was determined by the molybdivanadophosphoric acid spectrophotometric method (Greweling, 1966). Potassium and nitrogen were determined, respectively, by flame emission

Table II. Total Elemental of Sludge, Cabbage Grown Thereon, and Control Cabbage

element	element concentration (ppm, dry wt) in: sludge	control cabbage	sludge-grown cabbage
Al	48190	17	100
As	18	0.3	0.6
Au	0.3	0.004	0.003
B	10	24	28
Ba	288	3.4	12
Br	14	20	12
Ca	64170	17930	26100
Cd	95	0.1	5.3
Ce	94	0.3	0.4
Cl	2024	3860	3200
Co	3.4	0.2	0.3
Cr	748	0.6	1.2
Cs	1.3	0.1	0.02
Cu	878	4.1	21
Eu	3.0	0.02	0.2
Fe	8694	228	155
Hf	3.9	0.01	0.05
Hg	3.3	0.3	0.1
I	40	0.4	0.2
In	2.0	0.03	0.01
K	20740	29600	18400
La	21	0.2	0.07
Lu	0.2	0.01	0.01
Mg	5200	1610	1570
Mn	691	30	16
Mo	5.7	0.4	2.5
N	5500	23300	35400
Na	9582	472	3790
Ni	94	0.4	39
P	4100	3200	5300
Pb	303	1.1	3.5
Rb	74	37	10
Sb	8.3	0.2	0.1
Sc	1.5	0.004	0.01
Se	2.3	0.04	0.08
Sn	977	13	8
Sr	1370	6	30
Ta	1.0	0.05	0.02
Th	13	0.1	0.3
Ti	993	20	28
U	1.9	0.2	0.01
V	33	0.1	0.2
W	6.3	0.1	0.5
Yb	1.1	0.1	0.1
Zn	1418	17	224

Table III. Concentrations of Elements in the Complete Guinea Pig Diets Which Were Notably Higher in the Sludge-Grown Cabbage vs. the Control Group

diets[a]	parts per million, dry weight Cd	Cu	Ni	Pb	Zn
control cabbage	0.1	7.3	0.4	0.5	27
sludge-grown cabbage	2.6	14	23	1.8	120

[a] Diets contained 45% (dry weight) cabbage.

Table IV. Elements Which Deposited in Higher Concentrations in Particular Tissues of the Animals Fed the Sludge-Grown Cabbage vs. the Control Crop

tissue	parts per million, dry weight[a]				
	Cd	Cu	Ni	Pb	Zn
control kidney	6.34 ± 1.44	25.5 ± 4.8	0.18 ± 0.04	0.54 ± 0.04	95.2 ± 4.3
sludge kidney	28.9 ± 2.9[d]	47.9 ± 4.3[c]	1.72 ± 0.16[d]	0.60 ± 0.05	116.2 ± 3.3[c]
control liver	1.21 ± 0.31		0.07 ± 0.04	1.11 ± 0.08	85.9 ± 2.6
sludge liver	6.90 ± 0.90[d]		0.19 ± 0.06	2.48 ± 0.38[c]	98.9 ± 2.8[c]
control muscle	0.07 ± 0.00				39.3 ± 1.6
sludge muscle	0.30 ± 0.13				46.1 ± 2.0[b]
control spleen	0.26 ± 0.03	2.42 ± 0.20			
sludge spleen	0.48 ± 0.06[c]	2.82 ± 0.21			

[a] Mean ± standard error. [b] Significantly different ($p < 0.05$). [c] Significantly different ($p < 0.01$). [d] Significantly different ($p < 0.001$).

and the Kjeldahl method. The method of Peech et al. (1953) was used to measure pH. Polychlorinated biphenyls (PCBs) reported as Aroclor 1254 were determined in the sludge, cabbage, and guinea pig livers using electron-capture gas chromatography (Pesticide Analytical Manual, 1971) but with different gas chromatographic operating parameters. The gas chromatograph was a Tracor Model 220 equipped with a nickel-63 electron-capture detector. The column was 4 mm i.d., 6 ft long, and packed with 3% OV-17 on 100–120 mesh Gas-Chrom Q. The column temperature was 185 °C with nitrogen (60 cm^3/min) as the carrier gas and (40 cm^3/min) purge gas.

Comparison of means (Tables IV, V, VI, and VII) was accomplished by the Student's "t" test as described by Steel and Torrie (1960).

RESULTS AND DISCUSSION

The elemental analysis of subsamples of the sludge and cabbage are listed in Table II. Twenty-six elements were higher in concentration in the cabbage grown on sludge as compared to the control crop. Notable among these elements were Cd, Cu, Ni, Pb, and Zn which we had previously shown to be typically high in municipal sludges and can be absorbed by plants and subsequently deposited into tissues of animals fed these plants (Furr et al., 1976a,b). Concentrations of these five elements in the complete diets (containing 45% dry weight of cabbage) fed the guinea pigs are given in Table III.

The elements which deposited in higher concentrations in specific animal tissues are presented in Table IV. Cadmium, copper, nickel, and zinc in kidney, cadmium, lead, and zinc in liver, zinc in muscle, and cadmium in spleen were significantly higher in the guinea pigs fed the sludge-grown cabbage. Certain of the element data is missing in Table IV owing to lack of sample or analytical interference. With the organ tissues, the elemental concentrations are reported on a whole organ basis, but, for instance, Cd is known to concentrate particularly in the kidney cortex. 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. Elements such as Cd, Cu, Pb, and Zn readily concentrate in liver and kidneys of animals (Browning, 1969). Dietary Zn, as present in plants with Cd, has been reported to diminish deposition of Cd in tissues of rats (Welch et al., 1978), but the magnitude of this antagonistic effect would probably depend on the dietary Zn/Cd concentration ratio.

The average total feed intake and weight gain per animal for the 100-day feeding trial are given in Table V. No indication of toxicosis was observed in the sludge-grown cabbage fed animals.

The concentrations of PCBs found in the harvested cabbage, the prepared cabbage rations, and the livers of the guinea pigs fed the rations are given in Table VI. The average concentrations of PCBs in the livers of the guinea pigs fed the sludge-grown cabbage was significantly higher ($p < 0.01$) than that in the control animals. The uptake of PCBs by crops from soil or sludge-amended soil has been reported (Iwata et al., 1974; Curry, 1977).

Table V. Average Total Feed Intake and Weight Gain per Animal for the 100-Day Feeding Trial

treatment	average total feed intake, g	percent increase in body weight[a]
control cabbage	2567	138 ± 15
sludge-grown cabbage	2695	150 ± 12

[a] Mean ± standard error.

Table VI. Concentrations of PCBs in the Harvested Cabbage, the Cabbage-Containing Diets, and the Respective Guinea Pig Livers

treatment	PCBs, ppb dry weight		
	cabbage	cabbage ration	guinea pig liver[a]
control	106	81	892 ± 17
sludge grown	400	202	1194 ± 104[b]

[a] Mean ± standard error. [b] Significantly different ($p < 0.01$).

Table VII. Intestinal Aryl Hydrocarbon Hydroxylase Activity in the Guinea Pigs

treatment	intestinal AHH activity, pmol of product (mg of protein)$^{-1}$ min^{-1} [a]
control cabbage	7.5 ± 1.2
sludge-grown cabbage	17.2 ± 1.2[b]

[a] Mean ± standard error. [b] Significantly different ($p < 0.01$).

The intestinal AHH activity in the guinea pigs is presented in Table VII. The activity was 129% higher in the animals fed the sludge-grown cabbage. The presence of a somewhat higher concentration of PCBs in the livers of the guinea pigs fed the sludge-grown cabbage may have been partly responsible for inducing intestinal AHH enzyme activity in these animals. However, other unidentified foreign compounds, e.g., polycyclic aromatic hydrocarbons, deriving from the myriad of industrial wastes may have also been present in the sludge-grown crop and have contributed to this intestinal enzyme induction. Although Wattenberg (1971) had first shown that rats fed cabbage exhibited increased AHH intestinal activity perhaps by natural plant constituents, this present study indicates a significant elevation of intestinal activity in

animals fed sewage sludge-grown cabbage as compared to animals fed "normal" soil-grown plants. This highly inducible enzyme may function largely as a detoxication system, yet there may result an increased toxicity in animals and may activate procarcinogens (Parke, 1975). Induction of hepatic microsomal mixed-function oxidase activities has been recently reported in swine fed sewage sludge-fertilized corn (Hansen et al., 1976). Interestingly Cd inhibits microsomal induction (Becking, 1976), but Zn could alter this effect (Becking and Morrison, 1970).

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Caustic Waste Disposal on Lakeland Fine Sand. A Potential Landspreading Problem

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The hydroxides of sodium, potassium, and calcium, caustics used in the citrus industry, were studied in relation to their effects on percolation in Lakeland fine sand. Quantitative compositions of dried aerobic and sun-dried citrus sludges were compared with previously reported decomposition products of other plant materials. These studies show that a combination of humus substances and cations present in citrus waste water could be detrimental to percolation in Lakeland fine sand.

The water crisis in Florida (Carter, 1974), high energy costs, and increasing environmental regulation have made food processors aware of the economic and technical attractiveness of land disposal techniques (*Federal Register*, 1974). The citrus industry uses about 8.25×10^{10} gal of water (370 gal of water/box of fruit processed $\times$ 223×10^6 boxes of fruit processed in U.S. in 1973–1974) (Ratcliff, 1977) per production year, and liquid waste constitutes the industry's largest waste disposal problem.

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Soils receiving waste water must be capable of passing average rainfall plus the waste water. These soils and their associated flora and fauna work in a natural process that filters and decomposes waste products (McLellon, 1971). Several factors affect the permeability of soils and the capacity of associated flora and fauna to remove organic and inorganic matter. Many textbooks and other publications on soils report that certain hydrated cations, including sodium and potassium, decrease percolation in soils containing clay. Much work has been published on the extraction of aromatic acids and phenols from soil with alkaline solvents, including ethanolic sodium hydroxide at pH 11 (summarized by Haider, 1971). However, the effect on soil percolation of combinations of the humus materials and sodium hydroxide solutions has not been