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

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industrial sources may be taken up by plants, however. Many investigators have studied the extent of metal absorption by different plant species as a function of sludge application rate, soil type and pH, and other factors, and this work has been reviewed by Chaney (1973) and Page (1974).

Very little research has been done on possible transfer of metals from plants grown on sludge-amended soils to foraging animals. Furr et al. (1976) grew Swiss chard on a Sassafras silt loam soil at pH levels of 5.5 and 6.5 which had been amended with 100 dry tons/acre of municipal sewage sludge from the Blue Plains waste-water treatment plant in Washington, D.C. Concentrations of Sb, Cd, Mn, and Sn found in various tissues of guinea pigs fed the sludge-grown crops at 45% of their diet for 28 days were significantly higher than those in the corresponding tissues of guinea pigs fed control chard grown in the soil alone. In the work reported, Swiss chard was grown on soil treated with municipal sludges from Washington, D.C. and Baltimore, and the harvested material was fed to guinea pigs to study element deposition in plant and animal tissues.

EXPERIMENTAL SECTION

Plant growth studies were carried out on plots located at the Beltsville Agricultural Research Center, Beltsville, Md. The soil was a Woodstown silt loam, pH 5.5, and having a cation-exchange capacity of 5.9 mequiv/100 g. The treatments included (1) an anaerobically digested sludge from the Back River Water Pollution Control Plant in Baltimore, Md., applied at the rate of 56 dry metric tons/hectare, (2) an anaerobically digested sludge from the Blue Plains Water Pollution Control Plant in Washington, D.C., applied at the rate of 112 dry metric tons/hectare, and (3) the latter Blue Plains digested sludge composted with wood chips by the windrow method (Epstein and Wilson, 1975) and applied at the rate of 224 dry metric tons of the sieved compost per hectare. Composting sludge by incorporation of wood chips aids aeration and concomitant oxidation of possible phytotoxic organic constituents in it. The material was incorporated into the soil using rotary cultivation. The plots remained fallow for 4 weeks to permit further microbial degradation of organic constituents and leaching of excess salts. Dolomitic limestone was added to adjust the soil to pH 6.0. All plots were fertilized with potassium. Phosphorus and nitrogen fertilizer was used only on the control plot.

"Fordhook Giant" Swiss chard (*Beta vulgaris cicla*) was planted in the plots in early July 1974 in 30-in. rows. It was harvested in September. The leaves were washed by repeated 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. The dried material from each treatment was then incorporated 28% by weight into balanced diets for guinea pigs. In the case of the Swiss chard grown on Blue Plains sludge only 20% was included in the diet owing to insufficient plant material.

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. Four animals were fed each diet. The Swiss chard diets were gradually introduced to the animals, initially fed a pelleted commercial diet. After 1 week they were fed only the semipurified, Swiss chard diet for 80 days. These diets (see Table I) contained all known required vitamins and minerals with the Swiss chard 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 ether inhalation and tissues were taken and freeze-dried prior to elemental analysis.

Table I. Composition of Animals Diets

Constituent	Percent dry weight
L-Arginine	1.0
Potassium acetate	2.5
Magnesium oxide	0.5
Vitamin mixture	1.0
Choline chloride	0.4
Ascorbic acid	0.2
Vitamin B_{12}	40 μg/kg
Briggs salt mix[a]	6.0
Casein	28.0
Dextrin	14.6[b]
Sucrose	10.3[b]
Corn oil	7.5
Swiss chard	28.0[b]

[a] See Reid and Briggs (1953). [b] For the diet including Swiss chard grown on Blue Plains sludge-amended soil, the percentages of dextrin, sucrose, and Swiss chard in the diet were, respectively, 20.0, 12.9, and 20.0.

Samples of the sludge, Swiss chard and animal tissues were analyzed for 40 elements by nondestructive neutron activation analysis by the procedure described earlier (Furr et al., 1975). Following dry ashing the determination of Cd, Pb, and Zn was performed by conventional stripping voltammetry using a Princeton Model 174 polarographic analyzer (Gajan and Larry, 1972). Nickel was determined by furnace atomic absorption using a Perkin-Elmer Model 303 spectrophotometer equipped with an HGA-2000 furnace. Cadmium, Cu, Fe, Mn, Ni, Pb, and Zn were also determined in the sludge and plant material by conventional flame atomic absorption spectrophotometry. Boron was determined by the curcumin spectrophotometric method of Greweling (1966). Arsenic was determined by dry ashing (Evans and Bandemer, 1954) the samples, distilling arsine, and analysis using the silver diethyldithiocarbamate spectrophotometric method (Fisher Scientific Co., 1960). Selenium was determined by the fluorometric method of Olson (1969). Extractable metals in the soil and sludge-amended soils were determined using diethyltriaminepentacetic acid–triethanolamine solution (Follett and Lindsay, 1971).

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

RESULTS AND DISCUSSION

The results of elemental analysis of the soil and sludge materials are given in Table II. Nineteen elements were higher in total concentration in sludge materials than in the soil, notably Cd, Cu, Pb, and Zn. Table III lists the concentrations of Cd, Cu, Ni, and Zn extractable with diethyltriaminepentacetic acid–triethanolamine solution. The magnitude of release of these elements generally increases with decreasing soil pH as would be expected. The concentrations of elements in the Swiss chard from the various soil treatments are shown in Table IV. Most of the elements which showed higher total concentrations in the sludges as compared to the soil (Table II) also were higher in concentration in the respectively grown chard. Interestingly, the concentrations of Ba, Br, Cr, Fe, K, Mo, and Rb were higher and those of Cd, Ce, Co, Cu, Mg, Mn, Ni, Sn, and Zn were lower in the chard growing on the composted Blue Plains sludge as compared to the levels of those elements in the chard grown on the same uncomposted sludge treatment.

Table II. Total Elemental Content of Soil and Sludges

Element	Parts per million (dry weight) in: Soil	Back River sludge	Blue Plains sludge	Blue Plains compost
Al	25700	25800	25500	26600
As	14	12	15	17
Au	0.002	0.04	0.1	0.08
B	2.0	6.0	8.0	12
Ba	177	242	288	323
Br	6.4	7.0	7.4	13
Ca	1590	2040	1840	4200
Cd	4.5	14	20	6.7
Ce	74	89	94	87
Co	9.1	9.4	8.0	15
Cr	42	93	83	155
Cs	1.0	1.3	2.1	2.6
Cu	69	2120	640	250
Eu	0.9	0.8	0.8	0.9
Fe	11500	12180	14980	23000
Hf	20	22	17	15
Hg			0.8	1.5
I	1.6	2.3	1.2	3.8
In		1.8		
K	7420	7430	8370	7460
La	7	31	31	28
Lu	0.6	0.6	0.6	1.5
Mg	4060	2640	4150	3950
Mn	318	385	216	293
Mo	1.1	2.6	1.8	4.0
Na	1786	1777	1644	1612
Ni		340	50	370
Pb	16.3	1175	960	335
Rb	60	59	42	87
Sb	0.6	1.6	1.8	2.8
Sc	3.9	4.0	4.9	4.9
Se	14	8.0	12	16
Sm	25	25	26	24
Sn	55	106	35	19
Sr	53	61	148	89
Ta	1.0	1.1	1.5	0.8
Th	15	14	16	17
Ti	5350	5360	5270	5270
U	2.2	3.8	2.9	2.7
V	60	66	65	56
W	2.7	2.6	2.7	1.7
Yb	3.4	3.2	2.7	2.6
Zn	43	5460	2690	930

Table III. Soil pH and DTPA-TEA[a] Extractable Metals from the Sludge-Amended Soils

Soil treatment	Soil pH before planting	Soil pH at harvest	Parts per million dry weight: Cd	Cu	Ni	Zn
Control	6.2	6.6	0.02	0.8	0.4	1.6
Back River sludge	5.8	5.0	0.1	26	3.4	61
Blue Plains sludge	6.0	5.7	0.3	15	1.1	65
Blue Plains compost	6.7	6.7	0.2	12	1.7	29

[a] Diethyltriaminepentaacetic acid-triethanolamine.

Table V lists concentrations of elements as determined in the kidney of one replicate guinea pig from each of the treatments. The elements listed are those which showed higher concentrations (than controls) both in the sludge-grown chard and kidneys of the respective animals consuming that chard. The elements B, Cd, Ni, and Pb were determined by the specific methods previously outlined in liver and kidney of each of the replicated animals. Only nickel was found significantly ($p < 0.05$) higher in these organs for guinea pigs fed Swiss chard grown on the Back River sludge as compared to the other treatments (Table VI). Cadmium and lead were not

Table IV. Elemental Content of Swiss Chard

Element	ppm (dry wt) in chard cultured in soil amended with: Soil control	Back River sludge	Blue Plains sludge	Blue Plains compost
Al	80	88	70	73
As	0.0	0.0	0.0	0.0
Au	0.01	0.002	0.01	0.0002
B	12	25	29	30
Ba	16	24	20	40
Br	27	35	28	41
Ca	11600	16300	14700	15100
Cd	0.5	1.5	2.7	1.4
Ce	1.0	0.5	1.2	0.5
Co	0.4	0.8	2.2	1.1
Cr	0.5	0.4	1.8	2.4
Ca	0.1	0.1	0.3	0.3
Cu	11	23	22	18
Eu	0.1	0.2	0.3	0.4
Fe	135	169	229	298
Hf	0.1	0.1	0.04	0.2
Hg	0.2	0.1	0.1	<0.05
I	0.2	0.5	0.3	1.0
K	22800	17040	19550	38830
La	0.3	0.4	0.4	0.5
Lu	0.03	0.02	0.1	0.01
Mg	10600	8080	9690	5940
Mn	290	780	1900	780
Mo	0.2	0.8	0.2	1.0
Na	2295	3408	3480	4208
Ni	1.4	22	3.5	1.7
Pb	5.6	12.2	9.1	9.9
Rb	48	25	62	71
Sb	0.5	0.6	0.2	0.02
Sc	0.01	0.1	0.01	0.02
Se	0.03	0.04	0.03	0.04
Sm	0.1	0.2	1.3	0.2
Sn	27	26	78	11
Sr	11	57	34	37
Ta	0.1	0.1	0.04	0.1
Th	0.2	0.3	0.3	
Ti	58	34	31	26
V	0.3	0.4	0.4	0.5
W	0.02	0.3	0.2	0.7
Yb	0.2	0.1	0.1	0.2
Zn	70	950	580	257

Table V. Elements Whose Concentrations Were Elevated (Above Controls) Both in Sludge-Grown Chard and Kidneys of the Respectively Fed Guinea Pigs

Element	ppm (dry wt) in kidneys of guinea pigs fed Swiss chard grown on: Soil[a] (control)	Back River[a] sludge	Blue Plains[b] sludge	Blue Plains[a] compost
Br	10.7	16.6	11.1	13.0
Ca	391		473	668
Co	0.6	0.7	1.0	
Eu	0.2	0.4	0.4	0.3
Fe	340	444	710	542
Sr	18	46		71

[a] Swiss chard fed at 28% of diet for 80 days. [b] Swiss chard fed at 20% of diet for 80 days.

Table VI. Nickel in Liver and Kidney of the Guinea Pigs

Tissue	Parts per million (dry wt) in tissues[a] of guinea pigs fed Swiss chard grown on: Soil control	Back River sludge	Blue Plains sludge	Blue Plains compost
Kidney	0.15 ± 0.02	1.1 ± 0.24[b]	0.05 ± 0.01	0.15 ± 0.04
Liver	0.53 ± 0.06	3.0 ± 0.91[b]	0.48 ± 0.11	0.75 ± 0.19

[a] Average ± standard error of the mean. [b] Significantly higher ($p < 0.05$) than the respective tissue of guinea pigs on the other treatments.

Table VII. Cadmium and Lead in Liver and Kidney of the Guinea Pigs

Tissue	Parts per million (dry wt) in tissues[a] of guinea pigs fed Swiss chard grown on: Soil control	Back River sludge	Blue Plains sludge	Blue Plains compost
		Cadmium		
Kidney	14.9 ± 2.35	13.5 ± 2.40	14.9 ± 3.28	15.8 ± 3.63
Liver	3.13 ± 0.97	2.72 ± 0.68	2.74 ± 0.59	3.62 ± 1.02
		Lead		
Kidney	0.52 ± 0.11	0.54 ± 0.04	0.28 ± 0.05[b]	0.49 ± 0.07
Liver	3.37 ± 1.23	3.46 ± 1.12	2.63 ± 0.46	2.83 ± 0.28

[a] Average ± standard error of the mean. [b] Significantly lower ($p < 0.05$) than the respective tissue of guinea pigs on the other treatments.

significantly higher ($p > 0.05$) in kidney or liver of the guinea pigs fed any of the sludge-grown chards as compared to the control crop (Table VII). The elevated concentrations of zinc in the sludge-grown Swiss chards in this study may have contributed to lowering the deposition of cadmium in the animal tissues (Welch et al., 1978).

Animals from all treatments were healthy with average weight gains of 450 g, not statistically different ($p > 0.05$) from controls. Other elements such as Ba, Mn, and Zn which were higher in concentration in the sludge-grown chards vs. the control (Table IV) were not found elevated in the respective animal tissues probably because they may be poorly absorbed by animals (Browning, 1969). It was unexpected that nickel was increased in both livers and kidneys of guinea pigs fed the Back River sludge-grown chard.

This preliminary study, as well as our previous investigation (Furr et al., 1976), indicates that it is possible for numerous elements to concentrate in crops grown on municipal sludge-amended soils as well as in tissues of animals fed these crops as a portion of their total diet. They also illustrate the necessity and advantage of using multielement analysis procedures for investigating such element accumulation. Some advantage would appear to accrue from composting sludge prior to amending soil since the more toxic and phytotoxic elements such as Cd, Cu, Ni, and Zn appear to be rendered less available to subsequently grown plants. Much more research is necessary, however, to verify this effect.

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