Multielement Absorption by Crops Grown in Pots on Municipal Sludge-Amended Soil

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Beans, cabbage, carrots, millet, onions, potatoes, and tomatoes were grown in pots containing soil amended with 10% by weight of Milwaukee activated sludge. Forty-two elements were determined in the edible plant tissues and growth media by neutron activation, furnace atomic absorption, anodic stripping voltammetry, and other spectrophotometric methods. Nineteen elements were higher in total concentration in the sludge than in the soil. B, Br, Ca, Cd, Cu, Fe, Mg, Mn, Na, Ni, Se, and Zn were found at higher concentrations in at least three of the crops grown on the sludge-amended soil as compared to the control. Cadmium and zinc were found at particularly high concentrations in cabbage, millet, and onions. Yields of each crop increased markedly except those of cabbage and tomatoes which decreased in the sludge–soil mixture.

Satisfactory disposal of about 50 million tons (Hudson, 1968) of municipal sewage sludges produced annually in this country constitutes a major portion of the national solid waste disposal problem. Its use as a soil conditioner and source of certain nutrients in agriculture has been studied. Since municipal sludges may typically contain elevated levels of Zn, Cu, Cd, Pb, Cr, and other elements resulting largely from industrial wastes, edible crops growing on sludge-amended soils may absorb these elements. The extent of absorption depends on the nature of the plant and element, its concentration, soil pH, and other factors.

Previous investigations of this problem have been largely confined to a few of the most common elements such as those listed above in mainly forage crops and much of this work has been reviewed by Page (1974). However, depending on the spectrum of industries served, municipal sludge may contain virtually any element. Furthermore, it is common practice for suburban home owners to load and haul away municipal sludge from sewage treatment plants for use on lawns, ornamentals, and edible garden crops. The objective of the work reported here was to study the extent of absorption of 42 elements by plants representing major classes of edible garden crops grown on sludge-amended soil.

EXPERIMENTAL SECTION

Sludge from the city of Milwaukee was used. It was obtained commercially as "Milorganite". It is a heat-treated, waste-activated sludge with a fertilizer equivalent of 6-4-0% N–P_2O_5–K_2O and an ash content of 30.9%.

The soil was an Arkport fine sandy loam (pH 5.3) sampled near Ithaca, N.Y. It was air-dried and sifted through a 2-mm screen and mixed by quartering. Ten percent (w/w) of the dry sludge was thoroughly mixed with the soil (or 100 dry tons of sludge per acre) in a cement mixer. Soil mixed with 10% Canadian peat moss was used as the control. The pH values of the final mixtures were Milorganite-amended soil (5.3) and control soil (5.0). Cabbage was planted (see description below) and it germinated and grew but the yield was low (see Table III). The other crops showed early phytotoxicity or poor seed germination and therefore the sludge–soil mixture was potted and kept moist in the pot for 1 year prior to planting them. (Amending soil with freshly processed sludge and planting immediately may cause phytotoxicity owing to elevated salt concentrations and the presence of toxic elements such as As, Cu, Ni, and Zn or organic constituents such as industrial cutting oils and other constituents. Salts must be leached out and time allowed for decomposition of organic components by soil microorganisms.) The inhibitory effect on plant growth and seed germination from freshly produced sludges added to soil has been reported by Lunt (1953, 1959).

The crops used were: Tendercrop bush bean (*Phaseolus vulgaris*), Golden Acre cabbage (*Brassica oleracea* var. *capitata*), Scarlet Nantes carrot (*Daucus carota* var. *sativa*), Japanese millet (*Echinochloa crusgalli* var. *frumentacea*), Downing Yellow Sweet Spanish onion (*Allium cepa*), Katahdin potato (*Solanum tuberosum*), and Vendor tomato (*Lycopersicon esculentum*). All of the crops were grown in 9-in. plastic pots containing 6 kg of the soil mixtures except potatoes which were grown in 12-in. pots containing 12 kg. The number of plants grown in each pot were: bean, 2; cabbage, 1; carrot, 3; millet, 5; onion, 3; potato, 1; and tomato, 1. All treatments were replicated twice.

At the time of planting, 4 g of 0-20-0 (as P_2O_5) and 1 g of 0-0-60 (as K_2O) were thoroughly mixed into the top 3 in. of growth media in the 9-in. pots. Twice these weights of fertilizers were used in the 12-in. pots. All plants were fertilized once each week during the 11th, 12th, and 13th weeks of growth with a solution containing reagent grade KH_2PO_4 (0.001 M), KNO_3 (0.005 M), and $Ca(NO_3)_2$ (0.005 M); 250 ml was added to the 9-in. pots and 500 ml to the 12-in. pots. All plants were watered daily, care being taken to avoid splashing soil on the aerial portions of the plants.

At maturity the crops were harvested. At harvest only the edible plant portions were collected for analysis. In the case of millet this included the entire aerial portion of the plant (stems plus grain). Prior to analysis all crop portions were thoroughly rinsed with distilled water to remove adhering dust. Carrots, onions, and potatoes were thoroughly brushed, rinsed, and then peeled. The respective, replicated, edible plant portions were combined and subdivided by homogenizing in a blender or chopping in a food cutter with stainless steel surfaces. The food material was freeze-dried in polystyrene containers, mixed, and subsampled for analysis.

A second planting of cabbage was made in the same pots. After harvesting the first heads, the pots of soil or sludge–soil were kept moist in the greenhouse (unheated) during the fall and winter to simulate field practice. At the time of the second planting, the contents of each pot

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were dumped out, lumps broken up, and the material (plus partially decomposed roots) again placed in the respective pot, fertilized as before, and seeded.

Subsamples of soil, peat moss, sludge, and crop material were analyzed for 33 elements using nondestructive neutron activation analysis as previously described (Furr et al., 1975). For quantitative measurements, peak areas were compared to results obtained with known standards, usually well-known chemical compounds or pure elements, but on other occasions to documented samples. Recently, for example, well-known geologic materials and coal samples obtained from the Bureau of Standards have been processed with agreement well within the accepted range of variability being found for nearly every element detected. In order to ascertain that continued quality be maintained, groups of three compounds of selected elements were periodically processed with the identity of the elements being changed each time. In the group of test elements processed, nearest to the experiment reported here, i.e. Na, Mg, Al, Ti, Cr, Mn, Cu, Mo, Cd, Sn, Ta, W, U, Zn, Se, and Sb, no net variances exceeding 5% compared to the previous standard values were found and most were within 1–2%.

Cadmium, Pb, Zn, Cu, Ni, Cr, Se, B, and As were determined by other methods. The determination of Cd, Pb, and Zn was performed by dry ashing the samples up to 475 °C followed by analysis by conventional stripping voltammetry using a Princeton Applied Research Corp. Model 174 Polarographic analyzer (Gajan and Larry, 1972). Following dry ashing, Cu, Cr, and Ni were 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 Olsen (1969) employing wet digestion of the sample and measurement of the fluorescence of piazselenol resulting from reaction of Se with 2,3-diaminonaphthalene. Boron was determined in the growth medias and Swiss chard samples by the curcumin spectrophotometric procedure (Greweling, 1966). 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). Soil reaction (pH) was determined by the method of Peech et al. (1953). Recoveries of Cd, Pb, Zn, Cu, Ni, Cr, Se, B, and As added to control samples prior to ashing ranged from 80 to 95%.

RESULTS AND DISCUSSION

The results of elemental analysis of the various constituents in the growth media are listed in Table I. Nineteen elements were present at higher total concentrations in the sludge than in the soil. In Table II are listed the concentrations of those elements in the crops which were found at higher levels in at least three of the crops grown on sludge-amended soil as compared to the control. The elements Cd, Ni, and Se were higher in every crop grown on the sludge-treated soil and Mn was higher in all of the crops except carrots on the sludge treatment.

As noted earlier cabbage was grown as a second crop. As shown in Table II the concentrations of all of the listed elements except Cu and Na increased in the control cabbage during the second year as compared to those in the first year control. Also, the concentrations of all of the elements except B and Zn decreased in the sludge-grown cabbage during the second year as compared to those in the first year crop on the sludge treatment. Notably, whereas the concentrations of Fe, Mg, and Na were higher in the sludge-grown cabbage vs. the control during the first year, the reverse was true during the second year.

Table I. Total Elemental Analysis of Soil and Amendment Materials

Element	Element Concn (ppm, dry wt) in Soil	Peat moss	Milwaukee sludge
Al	39 400	252	11 900
As	2.9	0.1	22.8
Au	0.004	0.02	1.0
B	14	5	9
Ba	331	36	297
Br	3.5	37	25
Ca	3 410	5600	14 200
Cd	0.1	0.01	112
Ce	77		27
Cl	82	346	3 460
Co	9.3	1.4	3.9
Cr	17.3	0.3	6 015
Cs	2.6	0.5	0.6
Cu	47	0.5	647
Eu		0.03	
Fe	24 900	468	23 000
Hf	9		1.1
Hg	0.1	0.1	4.5
I	1.2	9.1	16.3
K	14 000	336	7 600
La	25	0.3	53
Lu	0.4	0.01	0.1
Mg	7 470	1190	5 770
Mn	278	4.3	254
Mo			30
Na	6 210	258	2 460
Ni	17	0.1	136
Pb	13	0.3	233
Rb	115		17
Sb	0.8	3.5	1.6
Sc	8.7	0.1	1.1
Se	0.3	0.2	1.8
Sm	5.0	0.02	2.5
Sn	12		73
Ta	0.6	0.1	0.2
Th	15	0.2	3.4
Ti	3 292	39	2 400
U	1.4	0.2	12.2
V	67	0.7	31.9
W	2.1	0.7	10
Yb	2.0	0.2	0.5
Zn	180	2.3	1 945

Analytical interferences prevented accurate analysis of Eu, Hf, Sm, and U in the plant material. The concentrations of the remaining elements in Table I were also determined in the crops but have not been included because the sludge treatment did not result in an increase in their absorption in more than two of the crops. It is possible that certain of the elements which did not increase in the edible plant parts as a result of the sludge amendment may have concentrated in other portions of the plants. While the elemental content of the peat moss was generally low the content of elements such as Sb may have contributed significantly to the total concentration of the element found in the control plants (0.4–2.5 ppm).

Cadmium reached very high concentrations in cabbage, millet, and onions. Cadmium is highly toxic with an oral LD_{50} of 88 mg/kg in rats for cadmium chloride (Christensen et al., 1975). Interestingly, zinc is also high in concentration in cabbage, millet, and onions grown on sludge–soil and zinc is known to have a marked protective effect on cadmium toxicity (Schroeder and Buckman, 1967). Nickel is much less toxic than cadmium with an oral LD_{50} of 1620 mg/kg in rats for nickel nitrate but both elements are believed to be carcinogenic. The observed increase in selenium in the plants grown on sludge–soil is small but probably significant since the method used (Olsen, 1969) is highly accurate, sensitive, and reproducible.

Table II. Element Concentrations (parts per Million, Dry Weight) in Edible Portions of Crops Grown in Pots on Sludge-Amended Soil

Element	Beans		Cabbage				Carrots		Millet		Onions		Potatoes		Tomatoes	
			Year 1		Year 2											
	Control soil	Sludge soil	Control soil	Sludge soil	Control soil	Sludge soil	Control soil	Sludge soil	Control soil	Sludge soil	Control soil	Sludge soil	Control soil	Sludge soil	Control soil	Sludge soil
B	16	34	23	4.6	35	35	19	21	17	12		19	6.0	6.0	4.0	19.4
Br	5.5	6.3	5.3	32	8.4	14.1	4.9	6.1	13	18	5.0	3.9	1.8	5.4	6.2	8.3
Ca	2670	3580	4500	14 900	7700	8140	1650	2210	3700	2290	4210	3490	237	223	780	1250
Cd	0.1	1.8	0.2	37.5	0.3	8.7	1.1	3.9	0.2	24.5	0.6	9.2	0.3	2.0	0.1	2.4
Cu	3.2	3.6	3.0	2.9	0.6	1.8	2.0	2.0	2.4	2.6	3.4	1.2	3.1	4.6	2.2	1.7
Fe	108	89	78	158	136	58	143	45	49	151	100	65	49	51	173	25
Mg	1340	1620	2160	5 740	2850	1440	730	760	3100	2970	1480	1020	740	830	880	1280
Mn	67	154	183	879	258	345	377	92	720	957	171	193	9.7	20	27	58
Na	47	35	2060	2 770	1850	838	907	1320	420	100	322	157	36	73	255	73
Ni	3.9	11.3	1.9	10.0	2.1	3.3	2.7	3.8	1.4	1.9	2.2	3.0	0.6	1.6	0.5	1.3
Se	0.02	0.04	0.01	0.11	0.02	0.04	0.00	0.03	0.02	0.03	0.00	0.03	0.01	0.04	0.01	0.02
Zn	44	79	68	1 086	204	1640	122	56	290	491	288	174	34	35	18.1	17.4

Table III. Yields of Edible Portion[a] of Crops in the Various Treatments

Crop	Av g (dry wt) per pot on the following treatments			
	Soil plus 10% peat moss (control)		Milorganite (10%) amended soil	
	Year 1	Year 2	Year 1	Year2
Beans	16.2		28.4	
Cabbage	45.5	23.4	3.3	30.8
Carrots	3.5		49.8	
Millet	107.0		130.0	
Onions	2.0		70.0	
Potatoes	78.5		177.0	
Tomatoes	39.5		33.9	

[a] Millet included stems plus grain.

Elements such as arsenic and chromium were notably higher in the sludge than the soil. Arsenic tends to be excluded by plants and chromium does not appear to be absorbed to a great extent either (Allaway, 1975). Mercury was also higher in sludge but it is tightly bound by humic substances especially those with sulfur and sulfhydryl functions (Page, 1974). Barium is possibly unavailable in soil owing to its precipitation as barium sulfate as has recently been shown for lead (Olson and Skogerboe, 1975).

The average yields in grams (dry weight) per pot are listed in Table III. During the first year, yields of each crop increased markedly except those of cabbage and tomatoes which decreased in the sludge–soil mixture. Conversely, the yield of cabbage cultured on the sludge treatment was greater than the control during the second year. The low yield of cabbage (first year) which as noted earlier was planted immediately in the sludge–soil medium may have been due to the presence of elevated concentrations of salt and other constituents in the sludge which affected growth and yield.

Many factors may determine the extent of absorption of elements by plants and their phytotoxicity to plants. As pointed out by Chaney (1973) these factors may include the nature, concentration, and valence of the element, soil pH, organic matter, and phosphate content, cation exchange capacity, and the nature and condition of the plant. Other factors such as redox potential, ionic competition, and chelation may also be important in controlling the availability of toxic as well as nutrient elements in sludge-amended soils.

Municipal sewage sludges may contain virtually any element depending on the spectrum of industries served (Furr et al., 1976a,b). Their composition varies not only with treatment plant location but with time as cities grow and industries relocate. Whereas the soil–plant relationships of some of the more common elemental contaminants in sludges can be roughly predicted, little is known about the soil chemistry or animal toxicity of many other elements which can be present. Indeed the concentration of antimony, cadmium, manganese, and tin in tissues of guinea pigs increased significantly over control animals when they were fed Swiss chard grown on sludge-amended soil (Furr et al., 1976b). Recommendations for the safe use of sludge in agriculture must therefore be preceded by absorption studies with many plants, extended animal feeding of harvested crops, and broad spectrum analytical methods for multielement determination in plant and animal tissues for correlation with possible pathologic symptoms.

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COMMUNICATIONS

N-Nitrosopyrrolidine Collected as a Volatile during Heat-Induced Formation in Nitrite-Containing Pork

Ground pork belly containing 200 ppm of sodium nitrite was heated to 177 °C and the condensate was collected and analyzed for *N*-nitrosopyrrolidine using gas chromatography and mass spectrometry. Nitrosopyrrolidine in an amount equivalent to 19 ppb (average) in the initial ground pork sample was detected in the condensate. When the total amount of nitrosopyrrolidine in the cooked pork, rendered fat, and condensate was determined, it was found that 20–40% of the total nitrosopyrrolidine produced was given off as a volatile during the cooking process. The amount of nitrosopyrrolidine produced was increased when 0.1% putrescine was added to the ground pork prior to heating. The addition of an equal amount of proline resulted in the production of even higher levels of nitrosopyrrolidine.

The occurrence of *N*-nitrosopyrrolidine in cooked bacon at levels up to 108 ppb has been reported (Crosby et al., 1972; Fazio et al., 1973; Sen et al., 1973). Proline, putrescine, and collagen, all naturally occurring in pork, have been shown to react with nitrite at high temperatures to produce nitrosopyrrolidine (Bills et al., 1973; Huxel et al., 1974; Gray and Dugan, 1975). Nitrosoproline, a compound which decarboxylates at bacon-frying temperature to yield nitrosopyrrolidine, has recently been isolated from raw bacon (Kushnir et al., 1975). Model systems consisting of heated oil (Bills et al., 1973; Pensabene et al., 1974; Kushnir et al., 1975) or high-temperature dry systems (Huxel et al., 1974; Gray and Dugan, 1975) have been used, rather than actual meat systems, to evaluate and compare various amines as nitrosopyrrolidine precursors. In addition, the study of nitrosopyrrolidine formation in heated bacon has involved analysis of cooked bacon and the rendered fat, but the possibility of nitrosopyrrolidine being given off as a volatile during the cooking process has not been investigated.

The purpose of this investigation was to compare proline and putrescine as nitrosopyrrolidine precursors in heated nitrite-containing pork and to determine the relative amount of nitrosopyrrolidine given off as a volatile during the cooking operation.

EXPERIMENTAL PROCEDURES

Sample Preparation. Two matched pork bellies were obtained fresh from a commercial processor. To ensure a homogenous sample for cooking experiments, the pork was ground, mixed, and reground with a Hobart meat grinder fitted with a $^3/_8$ in. plate. The ground pork was stored at −23 °C until used in the experiments. In preparation for cooking, sodium nitrite was mixed into the pork at a level of 0.02% (200 ppm). Putrescine dihydrochloride (K and K Laboratories, Inc.) at levels of 0.10 and 0.40% (calculated as putrescine) was incorporated in the same manner into portions of the pork containing 200 ppm of added sodium nitrite. Proline at a level of 0.10% was mixed into other portions of pork containing 200 ppm of added sodium nitrite. The pork was reground with a meat grinder at least three times to ensure adequate mixing of the added compounds.

Cooking Method. One hundred grams of the material was placed in a 500-ml boiling flask which was fitted with a distillation head, condenser, and receiver. Thermocouple