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compound, which has not been identified previously as a natural product.

From the *Cladosporium* bioassay on thin-layer plates, the minimal detectable amount is 3 μg for chavibetol acetate, 3 μg for chavibetol, 30 μg for chavicol, 10 μg for allylpyrocatechol diacetate, and 1 μg for allylpyrocatechol. The ED$_{50}$ for allylpyrocatechol in the *Pythium* bioassay is 30.5 μg/mL (0.203 μM). By comparison, the ED$_{50}$ for catechol in the same bioassay is 22.5 μg/mL (0.204 μM) and for eugenol is 33 μg/mL (0.201 μM).

P. betle has been reported to have anthelmintic activity (Chopra et al., 1950; Ali and Mehta, 1970), and although it takes substantial amounts (200 μg/mL each compound) of these pure isolates to produce complete mortality of the nematode *C. elegans*, the relatively high concentration of these compounds in the leaves would probably result in an effective anthelmintic dose.

Whereas antifungal activity of *P. betle* leaves and of its essential oil has been reported (Bangar et al., 1966), we have identified the active compounds and quantified their antifungal activity. These five compounds are by weight 0.97% of fresh leaves and 42% of the chloroform extract (Figure 1). Chavicol, chavibetol, and allylpyrocatechol are known natural compounds previously isolated from *P. betle* (Ueda and Sasaki, 1951); however, we think that chavibetol acetate and allylpyrocatechol diacetate are new natural compounds. The fungicidal activities of these new compounds are in the same range as the well-known natural fungicides eugenol and catechol, and their extraordinarily high level in the leaves of *P. betle* confers strong activity to the extract.

The common practice of chewing the leaves of *P. betle* may extract these allyl phenols and inhibit common opportunistic fungal pathogens such as *Candida albicans*, which can infect the oral mucosa. Since leaves of *P. betle* have been used as dressings for sores and wounds (Bangar et al., 1966), the compounds we have isolated may promote wound recovery through their antiseptic properties. It appears likely that some of the diverse biological effects of *P. betle* leaves and oil are due to these identified allylphenols and that these and similar allylphenols should be evaluated for medicinal and agricultural applications.

Registry No. 1, 12408-12-7; 2, 13620-82-1; 3, 501-92-8; 4, 501-92-8; 5, 1941-09-9.

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Total Glycoalkaloid and Mineral Content of Potatoes Grown in Soils Amended with Sewage Sludge

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The effect of sludge-amended soil on the total glycoalkaloid (TGA) and mineral content of Katahdin potatoes was investigated. In both years TGA content of potatoes grown on sludge-amended soil was not significantly different from that of controls. The tubers grown on sludge-amended soil were significantly lower in K content and higher in Mg content than that of controls. The B, Cd, Cu, Ni, and Zn content of potatoes increased significantly when grown on sludge-amended soil whereas Al and Na decreased. No significant trend was observed for the following minerals: Co, Cr, Fe, Mn, and Pb.

Vesilind (1980) projected that approximately 10^7 dry metric tons of municipal sludge will be produced annually in the United States by 1990, and the need to find safe methods for its disposal has led New York and other industrialized states to consider its potential use as a fertilizer and soil conditioner in agriculture (Boyd et al., 1982). However, there is equal concern that trace metals such as Cd, Cu, Ni, Pb, and Zn and refractory synthetic organic compounds such as polychlorinated biphenyls generally present in sludge (Furr et al., 1976) may be toxic to crops, animals, or man and/or the concentration of toxic substances present in crops may increase sufficiently to have deleterious effects on both animals and humans.

Considerable research is under way to study the possible use of sludge as fertilizer or as a soil conditioner in agriculture. On the beneficial side, it has been shown to increase soil organic matter and moisture-holding capacity (Hansen and Hinesly, 1979). Increases in potato tuber yield were found with municipal sewage sludge fertilization rates of 112, 225, and 450 metric tons (mt) per hectare (ha) compared to the control. Since sludge was low in K, all treatments received a preplant application of K (Dowdy

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and Larson, 1975). Potatoes grown on sludge-amended soil were shown to contain higher amounts of ascorbic acid, riboflavin, thiamin, and niacin on a dry weight basis, but no significant differences in flavor were observed (Lee et al., 1980).

Relatively little is known concerning the effect of sludge fertilization on potato tubers, especially glycoalkaloids, a group of naturally occurring toxic compounds. In commercially available tubers, 95–99% of the potato glycoalkaloid fraction consists of α-solanine and α-chaconine. These differ only in the sugar residues linked through the 3β-hydroxy group of solanidine, the parent aglycon. The glycoalkaloids are (1) potent cholinesterase inhibitors (Harris and Whittaker, 1962), (2) tetratogenic to various animals (Mun et al., 1975), and (3) the cause of several cases of poisoning of humans and farm animals (McMillan and Thompson, 1979; Bömer and Mattis, 1924). Potatoes having total glycoalkaloid (TGA) content exceeding 14 mg/100 g of fresh weight have been reported to impart bitter and undersirable flavor (Sinden et al., 1976). For release of new potato varieties in the United States, a level of 20 mg/100 g of fresh weight of tuber or higher is considered unacceptable (Jadhav and Salunkhe, 1975).

The TGA content in potatoes can vary depending upon variety, maturity, environmental factors, and mechanical- or chemical-induced stresses (Jadhav et al., 1981). Glycoalkaloids are concentrated in the cortex (including the periderm) with the concentration decreasing from outside to inside (Reeve et al., 1969).

Reports on the TGA content as influenced by fertilization practices have been confusing. Cronk et al. (1974) reported that in some varieties excessive application of nitrogen (202 kg/ha) significantly increased the TGA level over the controls receiving the normal level (135 kg/ha) of nitrogen. However, Nowacki et al. (1975) reported that increased nitrogen fertilization resulted in lower TGA levels. Boron application significantly increased TGA levels (Grechushnikov, 1955). Data collected in our laboratory indicated that TGA levels increased with Mg applications ($MgSO_4$) to the soil and application of zinc sulfate at a rate of 112 kg/ha (unpublished results).

Accumulation of trace metals in the tissue of edible vegetables grown on sludge-amended soils is well established. Edible crops grown on soils amended with sludge may absorb metals depending on the nature of the plant, the metal, the metal concentration, soil pH, and cation-exchange capacity (CEC) (Page, 1974). Cd, Zn, Cu, and Ni are important trace metals since they are concentrated in municipal sludge and are easily absorbed and translocated into many plants. Cadmium is of most concern because of its mammalian toxicity and deposition in animal tissues (Fox, 1979). Zinc is of interest since it exhibits protection against toxic effects of cadmium in animals (Browning, 1969).

Dowdy and Larson (1975) reported small, but significant, increases in the Zn and Cd content of edible fruit, tuber, and root tissue, whereas Cu and Pb remained unchanged. The ration of Cd to Zn in sludge was shown to affect Cd absorption by vegetables (Giordano and Mays, 1975).

The addition of sewage sludge to soil can either increase or decrease pH, which subsequently alters the availability of metals to plants. In general, the availability of Zn, Cu, Ni, Cd, and Pb in soils increases as soil pH decreases (Sommers, 1980).

The mineral content of potatoes has been attributed to differences in soil type, mineral content of the soil, and varietal differences (Augustin, 1975). The mineral content of cortex and pith tissues within the same tubers also differs and, in general, higher concentrations of minerals are found in the cortex region. Recently, in the United States there has been a significant increase in the consumption of potato peels in the form of fried peels and baked potato peels as well as French fries and chips in which the potatoes were not peeled before processing. Therefore, it is important to determine the effect of sewage sludge on the mineral content of cortex sections of potatoes. The effect of sewage sludge on TGA content of potatoes has not been studied, and it is important that this factor be considered.

The objectives of this study were to determine the effects of large amounts of sludge application on the total glycoalkaloid content as well as the overall mineral content of potatoes. Since the metabolic activity of cortex is considerably higher than that of pith tissue, this section of the tuber was selected for the analyses.

MATERIALS AND METHODS

Katahdin potatoes for the study were grown at Binghamton, NY, on a Mardin channery silt loam soil (coarse, loamy, mixed, mesic Typic Fragiocrept). The application site was established in 1975 as part of a commercial sewage sludge disposal operation. The sludge was derived from a treatment plant that treated effluents discharged by about 100 industries as well as domestic wastes.

The average composition of the anaerobically digested sludge was estimated to be total solids 4%, Kjeldahl nitrogen 5.5%, ammonia nitrogen 2.5%, phosphorus 2.0%, Cd 130 mg/kg, Cu 1050 mg/kg, Ni 540 mg/kg, Pb 545 mg/kg, and Zn 3500 mg/kg on a dry weight basis. However, sludge composition varied substantially during the application period. No lime or other chemicals were added during the treatment process. During the 5 years of use, the disposal site received sewage sludge at the rate of 100 to 120 dry mt/ha annually, which is considered a high rate of application. The total quantity of metals added to the soil was estimated to be Cd 7 kg/ha, Cu 580 kg/ha, Ni 300 kg/ha, Pb 300 kg/ha, and Zn 1920 kg/ha. Sewage sludge was applied by spray irrigation and by soil injection to a depth of 15 cm by a special all-terrain vehicle (Ag-Gater) with a delivery capacity of 45 000 L. The treated soil was later disked to a depth of 20 cm. No further sludge applications were made after 1979. Corn was grown on the site in 1980 growing season, and potatoes were grown in 1981. Potatoes (*Solanum tuberosum* L., cv. Katahdin) were grown on this site in a completely random design. The pH for year 1 (mean ± SD) was 5.3 ± 0.1 and for year 2 was 5.1 ± 0.1. Cation-exchange capacity (CEC) was 24.6 ± 2.4 for both years. Katahdin potatoes from the same seed source were grown on a control plot that had never received sludge but had received the usual amount of N–P–K (168 kg/ha NH_4NO_3–336 kg/ha P_2O_5–168 kg/ha K_2O) fertilizer each year. The pH of the control soil was 5.1. Potatoes were planted on June 10, 1981, and harvested Oct 9, 1981. In the following year, the planting date was May 26, 1982, and the harvesting date was Sept 28, 1982.

Samples of sludge-amended soil were collected and analyzed prior to growing season before potatoes were planted to establish existing levels of metals in the soil. Elemental analyses of the sludge-amended soil are presented in Table I. These were performed by the Soil Testing Laboratory of the Department of Agronomy at Cornell University (Greweling and Peech, 1965).

Potato tubers were washed, dried, and stored at 5 °C in the dark for 6 months prior to analysis. Potatoes of comparable size were selected in order to limit variations resulting from size differences. Tubers were cut longitudinally from bud to stem end in order to include both

Table I. Elemental Composition of Sewage Sludge Amended Soil[a]

element	concentration year 1	concentration year 2
	kg/ha[b]	
P	99.8 ± 11.2	90.4 ± 13.6
K	299.0 ± 28.2	232.2 ± 11.0
Mg	302.0 ± 29.9	198.1 ± 23.21
Ca	3416.0 ± 233.0	2772.0 ± 183.0
Mn	722.0 ± 174.0	662.7 ± 75.3
Fe	23.4 ± 2.91	62.4 ± 6.6
Al	42.0 ± 3.1	66.5 ± 5.5
Zn	494.0 ± 115	299.7 ± 32.7
	ppm on a Dry Weight Basis[c]	
Cu[d]	192.2 ± 21.8	161.8 ± 14.1
Cd	35.1 ± 4.7	31.5 ± 2.6
Zn	692.6 ± 83.1	640.0 ± 64.5
B[e]	2.2 ± 0.3	2.1 ± 0.3

[a] Mean ± SD of five determinations. [b] NaOAc/HOAc soluble (available). [c] Acid soluble by 8 N HNO_3 digestion ("total"). [d] Acid soluble by 0.1 N HCl digestion ("available"). [e] Water soluble ("available").

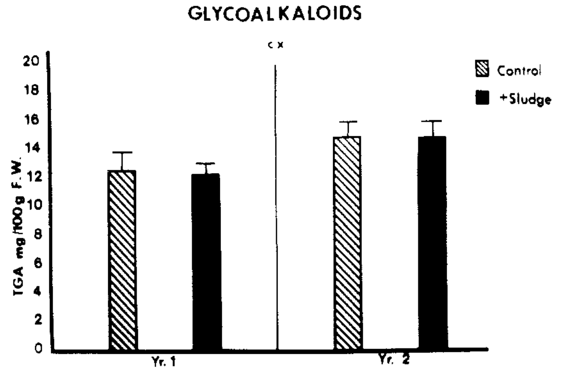

Figure 1. Effect of sludge-amended soil on the TGA content (fresh weight basis) of cortex tissue of potatoes.

apical (bud) and basal (stem) portions, and slices were subsequently separated into cortex and pith sections. Cortex tissue (including the periderm) was used in the study since this is the area highest in glycoalkaloid content as well as highest in metabolic activity. Most of the trace elements also accumulate in this area. Fresh cortex tissue from four tubers was used for each glycoalkaloid analysis. Cortex tissue from 10 tubers each from the control as well as treated was frozen, lyophilized in a Stokes freeze–dryer, ground in a Wiley Mill through a 40-mesh screen, and stored under nitrogen until analyzed.

Total Glycoalkaloid (TGA) Determination. Analysis of fresh tubers was made using the modified titration method of Bushway et al. (1980). Glycoalkaloids were extracted by using a chloroform–methanol bisolvent (Fitzpatrick and Osman, 1974) mixture (1:2 v/v), followed by precipitation of TGA in concentrated ammonium hydroxide, and quantified by nonaqueous titration. Four determinations were made on the control potatoes as well as those grown on the sludge-amended soil.

Determination of Minerals. Freeze-dried potato powder was analyzed for mineral content by atomic emission spectroscopy using an inductively coupled plasma system as described by Fassel and Kniseley (1974). Four determinations were made on controls and those that received sewage sludge.

Statistical Analysis. Completely random design was employed, and statistical significance of the data was determined by using 2 × 2 analysis of variance with protected LSD test described by Steel and Torrie (1980).

Table II. Average Precipitation (cm) and Temperature (°C) during 1981 and 1982 Growing Seasons

month	precipitation 1981	precipitation 1982	temperature 1981	temperature 1982
May		9.88		15.4
June	8.99	18.01	18.4	16.7
July	5.05	4.75	20.8	21.1
August	5.05	7.47	19.9	18.0
September	8.64	4.72	14.7	16.1
October	11.99	2.36	7.9	10.3

Table III. Macromineral Content (Percent on a Dry Weight Basis) of Cortex Tissue of Potatoes

element	mean[a] control	mean[a] +sludge	range control	range +sludge
year 1				
Ca	0.038 ± 0.002	0.048 ± 0.002	0.037–0.041	0.046–0.049
K	1.91 ± 0.06	1.68 ± 0.07	1.86–1.98	1.61–1.76
Mg	0.088 ± 0.010	0.090 ± 0.002	0.081–0.101	0.088–0.092
P	0.273 ± 0.030	0.216 ± 0.035	0.235–0.295	0.186–0.249
year 2				
Ca	0.034 ± 0.004	0.026 ± 0.001	0.031–0.039	0.026–0.027
K	1.89 ± 0.05	1.69 ± 0.03	1.84–1.92	1.66–1.72
Mg	0.090 ± 0.007	0.122 ± 0.001	0.085–0.098	0.121–0.123
P	0.229 ± 0.010	0.268 ± 0.003	0.219–0.239	0.265–0.270

[a] Mean ± standard deviation.

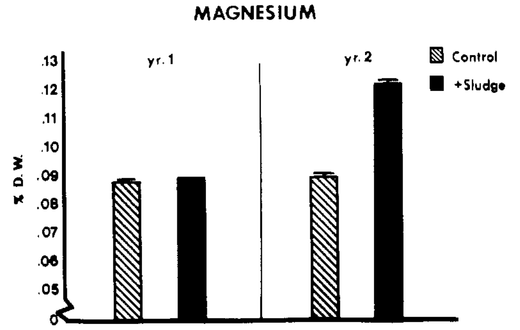

Figure 2. Comparison of magnesium content (% on a dry weight basis) of potatoes grown in sludge-amended soil.

RESULTS AND DISCUSSION

Total Glycoalkaloids. In both years the TGA content of potatoes grown on sludge-amended soil was not significantly different from that of controls (Figure 1), but the TGA content of potatoes in year 2 was significantly ($p < 0.05$) higher than in year 1 for both control and treated. This may have been due to variation in climatic conditions (Table II). Sludge-amended soil had high concentrations of phytotoxic minerals that could cause stress to the plants and possibly increase the TGA content of tubers. However, an increase was not observed.

Macrominerals. The macromineral content of potatoes is presented in Table III. In both years the K content of the tubers was significantly ($p < 0.05$) lower with sludge-amended soil, but the main factor (year) and interaction of treatment by year were not significantly different. The low K content in potatoes grown on sludge-amended soil is probably due to low K content in the sludge (Lee et al., 1980).

For Mg both the factors, treatment and year, were significant ($p < 0.05$). Also, the interaction of treatment by year was significant ($p < 0.05$). In both years the Mg content of the tubers was higher with sludge-amended soil (Figure 2), but only in year 2 was it highly significant ($p < 0.01$).

A significant increase ($p < 0.05$) in the Ca content of the tuber was found in year 1 with sludge-amended soil, but the opposite trend was observed in year 2. The Ca content

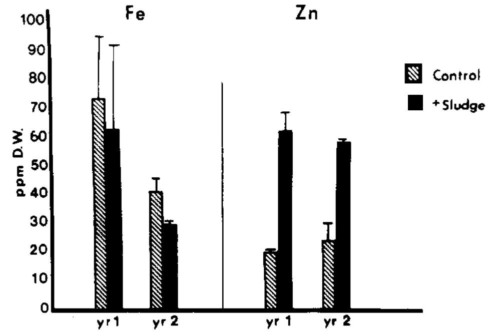

Figure 3. Comparison of the iron and zinc content (ppm on a dry weight basis) of potatoes grown in sludge-amended soil.

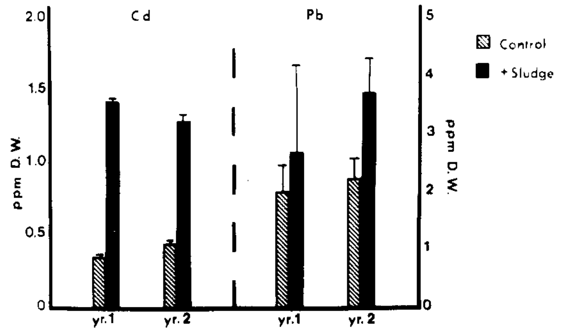

Figure 4. Effect of sludge-amended soil on the cadmium and lead content (ppm on a dry weight basis) of potatoes.

Table IV. Micromineral Content (ppm on a Dry Weight Basis) of Cortex Tissue of Potatoes

element	mean[a] control	mean[a] +sludge	range control	range +sludge
year 1				
Al	46.2 ± 10.1	33.3 ± 3.6	34.8–53.8	30.2–37.7
B	0.98 ± 0.23	7.26 ± 1.42	0.82–1.10	6.02–8.55
Cd	0.37 ± 0.04	1.44 ± 0.02	0.33–0.42	1.42–1.46
Co	0.55 ± 0.34	0.92 ± 0.29	0.34–0.94	0.59–1.28
Cr	1.36 ± 0.40	2.22 ± 1.12	0.91–1.70	1.25–3.38
Cu	9.29 ± 3.33	14.23 ± 4.39	5.56–12.00	8.50–19.20
Fe	74.4 ± 21.0	63.3 ± 29.1	50.3–88.8	38.4–95.6
Mn	7.40 ± 1.10	16.89 ± 8.40	6.72–8.68	9.52–24.90
Na	149.8 ± 13.0	83.2 ± 25.1	135.6–154.0	61.2–105.0
Ni	0.97 ± 0.41	12.44 ± 1.27	0.50–1.30	11.28–13.90
Pb	2.08 ± 0.45	2.76 ± 1.72	1.68–2.57	1.16–4.40
Zn	20.1 ± 0.7	62.9 ± 6.4	19.5–20.8	57.2–71.8
year 2				
Al	26.4 ± 4.3	20.7 ± 0.9	22.1–30.7	19.8–21.5
B	2.59 ± 0.78	7.70 ± 0.16	2.03–3.14	7.51–7.90
Cd	0.46 ± 0.10	1.13 ± 0.26	0.35–0.53	0.78–1.33
Co	0.89 ± 0.17	0.86 ± 0.33	0.69–1.01	0.42–1.12
Cr	1.35 ± 0.45	1.79 ± 0.20	0.86–1.73	1.60–1.99
Cu	7.80 ± 2.05	9.17 ± 0.21	5.44–9.15	8.97–9.45
Fe	41.5 ± 4.3	29.7 ± 0.7	37.7–46.2	28.7–30.3
Mn	8.25 ± 0.23	6.75 ± 0.12	8.06–8.17	6.61–6.89
Na	27.5 ± 11.9	18.3 ± 2.7	19.7–41.2	15.4–21.8
Ni	1.54 ± 0.95	12.90 ± 0.66	0.50–2.35	12.00–13.60
Pb	2.31 ± 0.36	3.82 ± 0.66	1.94–2.64	3.16–4.73
Zn	24.8 ± 6.1	59.1 ± 0.9	19.0–31.2	58.3–60.2

[a] Mean ± standard deviation.

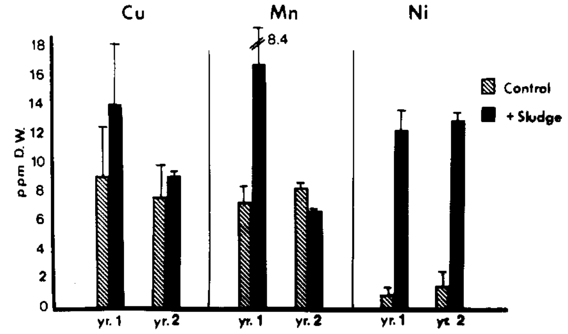

Figure 5. Effect of sludge-amended soil on the copper, manganese, and nickel content (ppm on a dry weight basis) of potatoes.

of the tubers for year 1 was significantly ($p < 0.05$) higher than for year 2. The interaction of treatment by year was significant ($p < 0.05$).

A significant increase ($p < 0.05$) in the tuber content of P was found in year 2 with sludge-amended soil, but no significant difference was found in year 1.

The interaction of treatment by year was significant ($p < 0.05$) for K, Mg, and Ca, indicating that the mineral interaction between K and Mg (Epstein, 1972), or K and Mg plus Ca (Grunes et al., 1968) may be equally important as environment and soil in mineral absorption by potato plants.

Microminerals. The concentration of trace metals in food is of major importance because of their toxicological and nutritional implications. When sewage sludge containing high amounts of metals is applied to soils, as was the case in this field experiment, the question arises as to whether the food (i.e., potato tubers) grown on the soil will take up enough of the metal to become toxic to humans. The micromineral composition of potato tubers is given in Table IV. In both years of study, the tubers grown on sludge-amended soil were significantly ($p < 0.05$) higher in concentrations of B, Cd, Cu , Ni, and Zn (Figures 3–5 and Table IV). However, in both years Al and Na were significantly ($p < 0.05$) lower in tubers grown on sludge-amended soil. Chromium was significantly higher ($p < 0.05$) in potatoes grown on sludge-ammended soil in year 2, and Mn did not show a definite pattern. Lead and Co were not significantly different due to treatment, but the Pb content was higher in potatoes grown on sludge-amended soil (Figure 4). The main factor year was significant ($p < 0.05$) for Al, Cd, Cu, Fe, Na, and Mn (Figures 3–5 and Table IV). All these minerals were higher in year 1 in the tubers than in year 2. Other minerals did not show year to year variation. The interaction of treatment by year was significant ($p < 0.05$) for Cd, Mn, Na, and Zn.

This trace metal accumulation is probably due to the availability of microminerals in sludge-amended soil. Since the main factors (treatment and year) and interaction of treatment by year were significant for most of the trace minerals, the uptake of these minerals must depend on their availability and environmental and soil factors (Sommers, 1980). Although Cd level in the sludge-amended soil was ~175 times that of the control soil (0.2 ppm on a dry weight basis), the Cd content of the potatoes grown in sludge-amended soil increased only 2-fold. Dowdy and Larson (1975) also found that the Zn, Cd, and Cu content of the tuber increased significantly when 450 mt/ha sludge was applied but observed only a small influence of sludge on the Pb content of potato tubers. Lee et al. (1980) reported that potatoes grown on sludge-amended soil were higher in sodium content. Liming an acid soil amended with sewage sludge decreased the Cd uptake by lettuce and chard (Mahler et al., 1978).

Davies and Crews (1983) reported that potatoes grown in soil contaminated by Pb/Zn smelter smoke accumulated Cd and Pb in the peel, which could possibly cause a health risk if large quantities of peel were consumed.

Wolnik et al. (1983) determined Pb and Cd levels for crops from nonpolluted areas so that these values could be used for evaluating the toxicological significance of consumption of these elements and their possible increase due to food processing. Levels of Cd and Pb in their study were generally lower than those previously reported. The reason for this could be that the authors used peeled potatoes instead of whole unpeeled potatoes.

Increased uptake of Cd by potatoes is of concern since FAO/WHO (1972) propose a 72 μg daily intake of Cd as maximum for adults. These levels are thought to provide a 3.5–6-fold margin of protection against an accumulation of 200 ppm of Cd in kidney cortex by age 50 (Kekwick, 1955). The U.S. food supply is now estimated to contain more than 90% of this Cd tolerance level (Fox, 1979). Therefore, a 2-fold increase in the Cd content of potatoes grown in sludge-amended soil, observed in this experiment, should be of concern to the consumer.

The largest increase in trace metal accumulation was observed for Ni. Both years potatoes grown on sludge-amended soil contained ~12 ppm of Ni when the control potatoes had only ~1 ppm. This indicates that the potato is an efficient accumulator of Ni under the experimental conditions these were grown. Nickel has a low rate of absorption and limited retention by animal tissue, and effects on animal production and health are unlikely (Underwood, 1971). Roach and Barclay (1946) reported that potato plants sprayed with solutions containing each of the following elements, B, Cu, Fe, Mn, Ni, and Zn, increased the yield.

CONCLUSION

The TGA content of potatoes grown on sludge-amended soil was not significantly different from that of controls. However, the potatoes grown on sludge-amended soil did affect significantly the trace metal content of the potato tuber. The B, Cd, Cu, Ni, and Zn content of potatoes increased significantly when grown on sludge-amended soil whereas Al and Na decreased. Cadmium uptake by potatoes is not directly proportional to the Cd level in the soil.

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Registry No. Ca, 7440-70-2; K, 7440-09-7; Mg, 7439-95-4; P, 7723-14-0; Al, 7429-90-5; B, 7440-42-8; Cd, 7440-43-9; Co, 7440-48-4; Cr, 7440-47-3; Cu, 7440-50-8; Fe, 7439-89-6; Mn, 7439-96-5; Na, 7440-23-5; Ni, 7440-02-0; Pb, 7439-92-1; Zn, 7440-66-6.

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