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Uptake of Selenium and Mutagens by Vegetables Grown in Fly Ash Containing Greenhouse Media

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Broccoli, endive, lettuce, onions, spinach, tomatoes, and perennial ryegrass were cultured in pots or pillow packs containing growth media that had been amended with increasing percentages of soft coal fly ash. The crops absorbed selenium in proportion to the percentage of fly ash in the growth media. Broccoli, onions, and lettuce absorbed the highest concentrations of selenium. The levels of selenium in ryegrass were maintained through five successive cuttings of the plants. Endogenous mutagens were found in endive, lettuce, and ryegrass. Endive and lettuce absorbed and translocated mutagens from the fly ash amended soil whereas ryegrass did not have this ability. A rat microsomal enzyme preparation was able to partially deactivate these mutagens to innocuous compounds.

Dietary selenium, at controlled levels above minimum requirements, has been correlated with anticarcinogenic properties in both laboratory animal studies and human epidemiologic investigations (Chortyk and Schlotzhauer, 1984). This property of selenium may be due to two of its known functions including its role in the active site of the enzyme glutathione peroxidase (Rotruk et al., 1973) and its interaction with toxic heavy metals. Glutathione peroxidase inactivates hydroperoxides and lipoperoxides, thus protecting cell constituents against free-radical damage (Tappel, 1978). Although the mechanism is not understood, selenium counteracts the toxicity of cadmium and mercury, possibly by promoting their accumulation in nontoxic forms (Frost and Lish, 1975).

The minimum requirement for selenium in man is estimated to range from 0.05 to 0.1 ppm in the diet. In many areas of the world, soils and also crops cultivated in these areas are deficient in selenium. Human selenium deficiency has been associated with Keshan disease in China where the soils have been shown to be deficient in selenium. The heart myopathy that develops in children raised in this area can be remedied in its early stages by

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dietary selenium supplementation (Chen et al., 1980).

Although more than 100 million tons of fly ash are produced in the United States annually (National Ash Association, 1978), only a small percentage is utilized by industry, mainly for road and building construction. Previous studies have shown that fly ash derived from coal-burning power plants can be used as a soil amendment to increase the uptake of selenium by crops (Furr et al., 1976, 1979; Gutenmann et al., 1979). As selenium is a micronutrient for humans, it is important that the use of fly ash as a selenium source in greenhouse growth media for the culture of vegetables be investigated. Concomitantly, the uptake of toxic compounds, which may be found in fly ash, needs to be investigated to ensure that these harmful components are not absorbed by plants subsequently consumed by man.

In this study, broccoli, endive, lettuce, onion, spinach, and tomato plants were grown to maturity in the greenhouse in growth media containing increasing levels of fly ash. The edible crop portions were analyzed for selenium. Ryegrass was also grown, and successive cuttings were analyzed as a measure of the sustained selenium absorption by a crop from a single application of fly ash. Since fly ash may also contain mutagenic compounds including polycyclic aromatic hydrocarbons (PAHs), certain of which can be taken up by plants (Fritz, 1983; Mueller, 1976; Edwards, 1983, 1986), the crops were also assessed for the possible presence of mutagens using the Ames (1983) *Salmonella typhimurium* assay.

EXPERIMENTAL SECTION

About 100 kg of freshly produced fly ash was obtained from Milliken Station, a coal-burning electric power generating plant owned by New York State Electric and Gas Corp. The plant is located in Lansing, NY, about 35 km north of Ithaca on the eastern shore of Cayuga Lake. This plant produces about 500 metric tons of fly ash/day from the combustion of bituminous coal, strip-mined mainly in Pennsylvania and to some extent in Maryland and Ohio. The fly ash was first mixed within itself and then into the growth media in a cement mixer. The pH of the fly ash as determined by the method of Peech et al. (1953) for soils was 4.5, and the concentration of selenium in it was 11.2 ppm.

The growth media into which the fly ash was incorporated in increasing percentages for each of the crops except onions was Cornell Peat-Lite Mix, a formulation used for seedlings or bedding plants, which consists of peat moss, vermiculite, lime, and fertilizer (Boodley and Sheldrake, 1977). The crops grown were Early One broccoli (*Brassica oleracea*), Buttercrunch lettuce (*Lactuca sativa*), Winter Bloomsdale spinach (*Spinacia oleracea*), Tiny Tim tomato (*Lycoperscon esculentum*), and Bison perennial ryegrass (*Lolium perenne*). Spartan Banner 80 onions (*Illium cepa*) were grown in 8-L plastic pots of fly ash containing Marly muck (*Terric medisaprists* in the Palms soil series) sampled near Oswego, NY (Table I). The crops were grown in plastic pots and pillow packs (Boodley and Sheldrake, 1966). Pillow packs were used as well as pots since they have become popular among the general public for the growth of crops in domestic greenhouses or under artificial lights. The pots were 18 cm in diameter and 13 cm in height. The pillow packs were made from polyethylene, 3 mil in thickness, using a heat sealer. They were 13 cm in diameter and 48 cm in length. Both pots and packs were provided with drainage holes. One plant was grown in each pot except for onions (three plants per pot) and ryegrass, which was planted as seeds (5 g of seed/pot). Since onions mature below ground, a deeper 8-L-capacity pot was required. Four plants were grown in each pillow pack. Each of the four were grown through a separate slit 5 cm long cut into the plastic along its length and evenly spaced about 10 cm apart. Each treatment was replicated in 10 pots and in 5 pillow packs. All plants were fertilized weekly 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). The volumes of fertilizer solution applied weekly were 60 mL for the 2-L pots and 250 mL for both the 8-L pots and packs. The composition, quantities, pH, and analyzed selenium content of the media are shown in Table I. All plants were watered as required.

Table I. Data Pertaining to Growth Media[a]

wt % fly ash in		g of media in			Se,[c] ppm
Cornell mix[b]	muck	pots	packs	pH	dry wt
0		578[d]	2020	6.4	0.08
6.0		655		6.4	0.59
11.2		733		6.3	1.14
20.2		889	3106	6.2	1.97
33.6		1044	3648	6.0	3.26
	0	2946[e]		7.7	1.60
	2.0	2966		7.6	1.83
	10.0	3046		7.4	3.18

[a] All media ingredients were prepared on a dry-weight basis. [b] Boodley and Sheldrake (1977). Also contained 1.38 wt% Sierrablend Nursery Mix (18–7–10 plus 1% iron) controlled release fertilizer (Sierra Chemical Co., Milpitas, CA). [c] As determined by fluorescence analysis. [d] 2-L pots. [e] 8-L pots used for onion culture.

At maturity, the entire edible portion of the plant was harvested for analysis. The ryegrass was harvested as five successive cuttings at 14-day intervals. The crops were air-dried except for tomatoes and onions, which were freeze-dried. The dried material was milled to a powdery consistency, mixed, and subsampled for analysis of selenium. 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 the reaction of selenium with 2,3-diaminonaphthalene.

Endive, lettuce, and ryegrass were analyzed for promutagens or direct-acting mutagens by an adaptation of the procedure of Ames (1983) following extraction of a subsample of the milled plants. A known weight of the dried plant was extracted with methylene chloride (50 mL/g of plant tissue) for 18 h in a Soxhlet apparatus. The extract was reduced in volume to about 3 mL in a rotary evaporator and then taken to dryness with nitrogen. The residue was then redissolved in 3 mL of dimethyl sulfoxide and stored at −70 °C until testing.

The preincubation modification of the standard *S. typhimurium* assay (McCann et al., 1975) as described by Yahagi et al. (1977) was utilized in the study. Briefly, a 0.1-mL aliquot of an overnight (16-h) culture of *S. typhimurium* TA 98 or TA 100 was added to 0.5 mL of 0.1 M potassium phosphate buffer, pH 7.4, and a 0.1-mL aliquot of the plant extract in DMSO for the measurement of direct-acting mutagens. The mixture was incubated for 20 min in a shaking water bath at 37 °C. A 2-mL aliquot of top agar containing 0.5 mM histidine/biotin was then added to the tube, mixed, and poured onto a Petri dish containing 25 mL of bottom agar. For the measurement of promutagens, a 0.5-mL aliquot of a 9000*g* (S9) supernatant liver fraction from Aroclor-pretreated rats was fortified with NADP, glucose 6-phosphate, and a $MgCl_2$/KCl mixture replaced the phosphate buffer used in the direct-acting assay (Maron and Ames, 1983).

The plates were incubated for 72 h at 37 °C, and the number of revertants was enumerated with use of a Bio-

Table II. Selenium Concentration in Crops Grown in Pots or Pillow Packs Containing Cornell Peat-Lite Mix A[a] with Increasing Percentages of Fly Ash

crop	Se,[b] ppm dry wt treatment (% fly ash)						
	0	2.0	6.0	10.0	11.2	20.2	33.6
			Pots				
broccoli	0.05 ± 0.00[w]		0.28 ± 0.03[x]		0.67 ± 0.03[y]	0.66 ± 0.03[y]	1.14 ± 0.09[z]
endive	0.03 ± 0.00[v]		0.12 ± 0.01[w]		0.23 ± 0.00[x]	0.42 ± 0.02[y]	0.69 ± 0.02[z]
lettuce	0.04 ± 0.00[v]		0.24 ± 0.01[w]		0.61 ± 0.04[x]	2.03 ± 0.15[y]	3.06 ± 0.19[z]
onion	0.02 ± 0.00[x]	0.27 ± 0.02[y]		1.69 ± 0.14[z]			
spinach	0.04 ± 0.00[v]		0.13 ± 0.00[w]		0.21 ± 0.01[x]	0.30 ± 0.03[y]	0.40 ± 0.03[z]
tomato	0.02 ± 0.00[v]		0.05 ± 0.00[w]		0.10 ± 0.00[x]	0.35 ± 0.01[y]	0.58 ± 0.03[z]
ryegrass							
1st cutting	0.01 ± 0.00[x]					0.86 ± 0.04[y]	1.30 ± 0.05[z]
2nd cutting	0.02 ± 0.00[x]					0.57 ± 0.03[y]	1.05 ± 0.04[z]
3rd cutting	0.02 ± 0.00[x]					0.52 ± 0.03[y]	1.39 ± 0.09[z]
4th cutting	0.05 ± 0.00[x]					0.85 ± 0.06[y]	1.98 ± 0.07[z]
5th cutting	0.03 ± 0.00[x]					0.78 ± 0.03[y]	1.63 ± 0.05[z]
			Packs				
broccoli	0.05 ± 0.00[x]					2.12 ± 0.16[y]	3.58 ± 0.20[z]
endive	0.03 ± 0.01[x]					0.60 ± 0.02[y]	1.12 ± 0.01[z]
lettuce	0.04 ± 0.00[x]					1.74 ± 0.19[y]	3.19 ± 0.54[z]
spinach	0.02 ± 0.00[x]					0.31 ± 0.02[y]	0.46 ± 0.04[z]
tomato	0.01 ± 0.00[x]					0.54 ± 0.01[y]	0.88 ± 0.02[z]

[a] Onions were grown in muck soil and fly ash. [b] Mean ± standard error for 10 replicated pots or 5 replicated packs. Dissimilar letter superscripts indicate significant difference ($p < 0.01$) between respective treatment means by Duncan's multiple-range test (Steel and Torrie, 1960).

tran colony counter. The background reversion rate (negative control) was determined in the presence of DMSO. Sodium azide (10 μg/plate) and 2-nitrofluorene (5 μg/plate) were used as the positive control substrates for TA 100 and TA 98, respectively, in the absence of S9, whereas 2-aminofluorene (10 μg/plate) was utilized for both strains in the presence of S9.

RESULTS AND DISCUSSION

The concentrations of selenium in the crops grown in pots and packs containing Cornell Peat-Lite Mix amended with increasing weight percentages of fly ash are shown in Table II. In all instances with the exception of broccoli grown in pots containing 11.2 vs 20.2% fly ash, the uptake of selenium by the crops was proportional to the percentage of fly ash in the growth medium. A similar observation was made in an earlier study with several vegetables grown in a potted soil containing increasing percentages of fly ash (Furr et al., 1976). Furthermore, cabbage grown in a potted soil amended with the same percentage of fly ash from 15 different locations in the United States absorbed selenium in proportion to its concentration (1.2–17 ppm) in the particular fly ash (Furr et al., 1977). This finding is of importance since the fly ashes were derived from different coal sources and were produced by coal-burning combustion units of differing design and operating parameters. It would appear, therefore, that the availability of selenium in fly ash for plant absorption may be quite independent of the coal source or precise conditions of coal combustion during fly ash production. Rather it may depend on the concentration of the element in a water-soluble form in the fly ash.

The concentration of selenium was maintained at an elevated level (0.5–2.0 ppm) through five cuttings of ryegrass. It thus appears that one application of fly ash can supply selenium continuously over an extended period of cropping. This sustained release and uptake of selenium had been reported earlier with a number of vegetables grown repeatedly in the same potted soils amended with fly ash (Furr et al., 1976) and with five successive cuttings of three grasses grown in the field on a fly ash amended soil (Gutenmann et al., 1979). Deep-rooted legume crops such as alfalfa and birdsfoot trefoil do not show this sustained uptake in the field since their roots eventually penetrate below the plow layer of soil, into which the fly ash is incorporated. This continued release of selenium to grasses resulting from one application of fly ash to the soil is very important from a practical standpoint for farm animals fed grass hay or silage or those grazing grass–legume mixtures.

Higher concentrations of selenium were consistently found in broccoli, onions, and lettuce, whereas spinach and tomato had the lowest levels. In most instances, the plants grown in the fly ash containing media in the pillow packs were higher in selenium than the corresponding plants grown in pots. This may be due to the ability of plant roots in the packs to exploit a greater volume of the growth media than those in the pots. The absorption of relatively higher concentrations of selenium by onions grown in the muck soil containing low percentages of fly ash as compared to the vegetables grown in the peat moss–vermiculite mixture with higher levels of selenium may have been due to the higher pH of the former medium (Table I). Selenium is generally more available for plant absorption at higher soil pH values.

On a parts per billion dry-weight basis, the selenium content of foods in the American diet has been reported to be fruits (2–13), vegetables (4–39), and meats (116–432) (Morris and Levander, 1970). Grains and their byproducts range from about 100 to 800 ppb selenium (Ferretti and Levander, 1974). Meats and grains are thus a better source of selenium than fruits and vegetables. Of interest, however, are reports that milling and processing grains (Ferretti and Levander, 1974), dry-heating cereals (Higgs et al., 1972), or boiling some vegetables result in a significant loss of selenium.

The estimated daily intake of selenium for humans in most parts of the world has been reported as 4–35 μg for infants and 60–250 μg for adults (Lo and Sandi, 1980). A concentration of 0.1 ppm of selenium on a fresh-weight basis may now legally be incorporated into rations for most farm animals in the United States to meet their dietary requirements. This level is considered to be too low by some researchers owing to the antagonistic effects of dietary sulfur, stress, and other factors (Rosenfeld and Beath, 1964). If the minimum daily intake of selenium in human

Table III. Crop Yields from the Various Treatments

crop[b]	yield,[a] g dry wt treatment (% fly ash) 0	2.0	6.0	10.0	11.2	20.2	33.6
			Pots				
broccoli	7.08 ± 0.98[x]		5.26 ± 0.66[x]		5.16 ± 0.20[x]	7.37 ± 1.00[x]	5.94 ± 0.70[x]
endive	4.68 ± 0.24[x]		10.71 ± 1.00[y]		9.36 ± 0.43[y]	5.53 ± 0.22[x]	5.05 ± 0.21[x]
lettuce	4.45 ± 0.28[x]		7.70 ± 0.52[z]		6.07 ± 0.56[yz]	5.58 ± 0.33[y]	5.19 ± 0.35[xy]
onion	233.9 ± 11.2[x]	241.9 ± 16.0[x]		225.8 ± 15.8[x]			
spinach	10.38 ± 1.04[x]		6.21 ± 0.62[y]		5.48 ± 0.52[y]	9.35 ± 1.02[x]	8.90 ± 1.02[x]
ryegrass							
1st cutting	8.96 ± 0.63[x]					10.61 ± 0.60[x]	10.16 ± 0.47[x]
2nd cutting	6.02 ± 1.11[x]					6.49 ± 0.23[x]	6.40 ± 0.15[x]
3rd cutting	11.27 ± 0.35[x]					13.88 ± 0.26[y]	13.37 ± 0.40[y]
4th cutting	5.64 ± 0.32[x]					7.30 ± 0.19[y]	8.27 ± 0.38[z]
5th cutting	5.23 ± 0.20[x]					5.44 ± 0.15[x]	5.67 ± 0.24[x]
			Packs				
broccoli	8.80 ± 0.49[x]					12.01 ± 1.51[x]	10.34 ± 0.52[x]
endive	18.29 ± 0.77[xy]					20.20 ± 0.76 [x]	17.37 ± 0.47[y]
lettuce	10.57 ± 1.55[x]					19.07 ± 1.04[y]	20.14 ± 1.38[y]
spinach	28.31 ± 3.46[x]					27.52 ± 2.28[x]	33.48 ± 1.72[x]

[a] Mean ± standard error for 10 replicated pots or 5 replicated packs. Dissimilar letter superscripts indicate significant differences ($p < 0.05$) between respective treatment means by Duncan's multiple-range test (Steel and Torrie, 1960). [b] Tomato yield was not measured.

Table IV. Mutagenicity of Extracts of Ryegrass, Endive, and Lettuce Grown on Fly Ash to *S. typhimurium* TA 98

plant	treatment	revertants[a] no S9	S9 added
ryegrass	control	596 ± 45[x] (4)	34 ± 5[x] (6)
ryegrass	33.6% fly ash	692 ± 106[x] (4)	40 ± 1[x] (6)
endive	control	342 ± 13[x] (5)	127 ± 6[x] (5)
endive	33.6% fly ash	407 ± 31[x] (6)	279 ± 22[y]* (6)
lettuce	control	601 ± 5[x] (5)	190 ± 22[x] (5)
lettuce	33.6% fly ash	769 ± 21[y]* (5)	488 ± 51[y]** (4)
	neg control	28 ± 1[b] (10)	29 ± 2 (10)
	pos control	378 ± 18[c] (10)	1632 ± 30[d] (10)

[a] Number of revertants ± standard error in at least two experiments. Dissimilar letter superscripts (x and y) indicate significant differences ($p < 0.05$* or $p < 0.001$**) between respective treatment means by Student's *t*-test (Steel and Torrie, 1960). The numbers in parentheses refer to the number of plates from which the results were obtained. [b] Number of revertants in the presence of dimethyl sulfoxide (solvent). [c] 5.0 μg of 1-nitrofluorene/plate. [d] 10.0 μg of 2-aminofluorene was used in the presence of a rat postmitochondrial supernatant (S9).

Table V. Mutagenicity of Extracts of Ryegrass, Endive, and Lettuce Grown on Fly Ash to *S. typhimurium* TA 100

plant	treatment	revertants[a] no S9	S9 added
ryegrass	control	138 ± 8[x] (4)	87 ± 5[x] (4)
ryegrass	33.6% fly ash	166 ± 7[x] (8)	95 ± 7[x] (6)
endive	control	551 ± 65[x] (6)	147 ± 18[x] (4)
endive	33.6% fly ash	598 ± 17[x] (5)	294 ± 48[x] (4)
lettuce	control	619 ± 47[x] (4)	292 ± 8[x] (4)
lettuce	33.6% fly ash	864 ± 49[y] (4)	307 ± 39[x] (4)
	neg control	97 ± 1[b] (9)	91 ± 3 (9)
	pos control	1181 ± 92[c] (7)	763 ± 67[d] (7)

[a] Number of revertants ± standard error in at least two experiments. Dissimilar letter superscripts (x and y) indicate significant differences ($p < 0.01$) between respective treatment means by Student's *t*-test (Steel and Torrie, 1960). The numbers in parentheses refer to the number of plates from which the results were obtained. [b] Number of revertants in the presence of dimethyl sulfoxide (solvent). [c] 10.0 μg sodium azide/plate. [d] 10.0 μg of 2-aminofluorene was used in the presence of rat postmitochondrial supernatant (S9).

diets is in the 0.1 ppm range, the vegetables grown at the 33.6% fly ash incorporation rate in this study would most closely approximate this selenium requirement on a fresh-weight basis. From 50 to 200 μg of selenium/day has been suggested as the requirement for human adults by the U.S. Food and Nutrition Board (1980). A number of other factors may influence the recommended daily intake including lean body mass, historical selenium intake (Levander and Morris, 1984), and the sparing effect of vitamin E on selenium deficiency.

The yields of the crops from the various treatments are given in Table III. There was no consistent effect of treatment on crop yield although optimal yields were obtained for endive and lettuce when the fly ash inclusion rate was 6–11.2% in pots and 20.2–33.6% for lettuce in packs. None of the crops exhibited external symptoms of phytotoxicity.

Evaluation of endive, lettuce, and ryegrass for mutagens is shown in Tables IV and V where it was demonstrated that both endogenous mutagens and absorbed mutagens were detected. To assess the uptake of mutagenic compounds from fly ash, an increase in the mutagenic response by extracts of plants grown in the presence of fly ash vs plants grown in the absence of fly ash must be observed. In this study, a statistically enhanced mutagenic response was observed in lettuce grown on fly ash amended soil, indicating that this plant was able to absorb and translocate mutagens from fly ash to its edible portions. The finding that endogenous mutagens were present in lettuce, endive, and ryegrass was not unexpected. Mutagens have been found in pepper, mushrooms, celery, rhubarb, herbal teas (Ames, 1983), and coffee (Shane et al., 1987). Naturally, the concentration of a benchmark PAH and promutagen, benzo[*a*]pyrene, in endive and lettuce has been shown to range from 12 to 50 ppb (Graf and Diehl, 1966).

With the addition of a rodent microsomal enzyme preparation containing both detoxifying and activating enzymes, the mutagenic response decreased indicating that natural or direct-acting mutagens were being detoxified to nonmutagenic components. As the percentage of deactivation by the microsomal enzymes on the extracts from lettuce and endive grown in the presence of fly ash was lower than plants grown on control media, it is postulated that promutagens absorbed by these plants were being activated to frameshift mutagens (Table IV) by the microsomal enzymes. As certain promutagens including benzo[*a*]pyrene are mutagenic only after activation by a microsomal enzyme preparation, it is possible that promutagens may have been absorbed by the plants. A suggestion that one of these promutagens is benzo[*a*]pyrene is unlikely as this compound has been shown to be absorbed extremely slowly by most plants (Mueller, 1976;

Borneff et al., 1973; Blum and Swarbrick, 1977). In addition, studies on the elution of benzo[a]pyrene from fly ash has shown that this PAH is eluted very slowly by organic solvents and aqueous solutions from fly ash particles (Soltys et al., 1986).

Uptake of PAHs and their translocation to the leaves of plants occurs at a very slow rate. Green beans, cantaloupes, and cottonseed absorbed and translocated less than 5 ppb of a [^{14}C]benzo[a]pyrene solution when grown hydroponically (Blum and Swarbrick, 1977). A more recent study in which bush bean plants were exposed to [^{14}C]anthracene showed that more than 60% of the radiolabeled PAH was incorporated into the roots, whereas only 39% was found in the stems and leaves (Edwards, 1986). Interestingly, about 50% of the anthracene absorbed was metabolized to more polar compounds by the plant. These studies indicate that different plants may absorb different PAHs at various rates. In addition, these findings were obtained with pure PAHs added to soils and not PAHs adsorbed to fly ash. In the present study, lettuce absorbed and translocated more mutagenic components from the fly ash than did either endive or ryegrass. The composition of the mutagens absorbed by the plants was not, however, determined. Desorption and uptake by plants of PAHs and other organic pollutants from fly ash has received little attention. A recent study by Pfeiffer and Kunte (1984) indicated that PAHs from sewage sludge were not absorbed by grape plants to any extent. The uptake of benzo[a]pyrene by radishes, carrots, and spinach appeared to depend on the physical characteristics of the soil in which these plants were grown as well as the concentration of the PAH (Mueller, 1976; Borneff et al., 1973).

Results obtained in this study indicate that fly ash produced as a byproduct in coal-burning electric power generating facilities can be added to plant growth media at an inclusion rate up to 33.6% as a source of selenium. However, uptake of mutagenic compounds by certain plant species from the fly ash also occurs. The effect of such mutagens on human health is unknown, as a 50% increase in the mutagenic response was obtained due to translocation of these components to the edible portions of endive and lettuce, but more than 40% of the mutagens were inactivated by a hepatic microsomal enzyme preparation. Further studies on the identification of mutagens absorbed by edible plants from fly ash needs to be undertaken.

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Fluorescence Detection of Phototoxic Psoralens in Vegetable Products

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This work describes the fluorescence detection of phototoxic psoralens in various vegetable products including celery, lime, carrot, parsley, and spinach. The spectral properties of psoralen, 5-methoxypsoralen, and 8-methoxypsoralen and raw extracts of celery and carrots are discussed in detail. The measurements used a portable fiberoptics luminoscope developed for monitoring surface and skin contamination.

I. INTRODUCTION

Recently there has been increasing interest in psoralens (linear furanocoumarins) because of their photobiological properties. DNA lesions caused by psoralen or its derivatives in conjunction with long-wavelength ultraviolet (UV) radiation have been investigated (Hearst, 1981). Psoralens were found to intercalate at two adjacent base pairs of DNA and form covalent bonds to a pyrimidine base on each DNA strand upon UV radiation, thus generating a cross-link. Psoralens, especially 8-methoxypsoralen (8-MOP), have been used in the treatment of various skin diseases such as psoriasis (Parrish et al.,-1974) and leukoderma (Scott et al., 1976). Psoralens occur naturally in a number of plants from several families and account for the phototoxicity associated with various species such as celery and plants such as parsnip roots (Beier and Oertli, 1983; Oertli et al., 1984). Chromatographic techniques have been developed to analyze linear furanocoumarins in celery (Beier et al., 1983; Beier, 1985). A recent study has investigated dermatitis in grocery workers associated with high concentrations of furanocoumarine in celery (Berkley et al., 1986; Seligman et al., 1987). Detection of psoralens in human foods is a critical step in assessing the importance of human exposure to phototoxic psoralens. This paper reports results from fluorescence measurements designed to detect psoralens directly in food plant liquid extracts. Emphasis is on surface and skin contamination of workers who are frequently involved in routine handling of vegetables or food plants. A fiberoptics luminoscope developed for monitoring skin contamination of workers in various industrial settings (Vo-Dinh and Gammage, 1981; Vo-Dinh, 1987) was used to measure the surface fluorescence of various products from food plants. Spectral characteristics of psoralen, 5-methoxypsoralen (5-MOP), and 8-MOP and raw extracts of celery and carrots have been investigated and will be discussed in detail. The results suggest that the use of direct fluorescence detection with the luminoscope has great potential for assessing the problems associated with skin contamination by phototoxic psoralens contained in food plants and vegetables.

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II. EXPERIMENTAL SECTION

2.1. Fiberoptics Luminoscope. A hand-held fiberoptics-based luminoscope was developed to conduct investigation of skin contamination among workers in energy technologies (Vo-Dinh, 1987). This instrument was further developed with extended capabilities for quantitatively measuring luminescent contaminants on skin or on solid surfaces. The detailed design and electronic circuitry of an improved version are given elsewhere (Vo-Dinh, 1987; Vo-Dinh and White, 1986). A field-portable version of this instrument is commercially available from Environmental Systems Corp., Knoxville, TN 37912. Only the basic features of the instrument are given here. The excitation source was a 125-W mercury lamp commercially available (PBL Electro-Optics, Inc., Model Hg-125). The filter holder, which accommodated an easily interchangeable interference filter with transmission in the UV, was attached to the entrance aperture of a bifurcated fiberoptics waveguide (Ealing Corp.) that was used to channel the exiting light onto the surface being scanned and to retransmit the luminescence of the contaminants back onto the entrance slit of the detection system. In this new instrument a holographic monochromator was used to select the spectral region for detection. Luminescence signals were detected by a photomultiplier (Research Support Instruments, Inc.) that operated in the digital single-photon counting mode. The signal recording, background-nulling, and data conversion system was designed and fabricated at Oak Ridge National Laboratory (ORNL).

2.2. Reagents and Materials. Linear furanocoumarins used in this study were obtained from the following sources: psoralen, Interchem. Corp., Paramus, NJ; 5-MOP, Aldrich Chemical Co., Inc., Milwaukee, WI; 8-MOP, Biochemical Laboratories, Redondo Beach, CA. Crystalline psoralen, 5-methoxypsoralen, and 8-methoxypsoralen were provided by NIOSH. Spectroscopic grade ethanol was used to prepare solutions of these compounds. Fluores-

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