Effect of Fertilization with Municipal Sludge on the Glutathione, Polyamine, and Cadmium Content of Cole Crops and Associated Loopers (*Trichoplusia ni*)

Patrick R. Hughes, Leonard H. Weinstein, Scott H. Wettlaufer, John J. Chiment, George J. Doss, Thomas W. Culliney, Walter H. Gutenmann, Carl A. Bache, and Donald J. Lisk*

Cabbage and collards grown in pots in municipal sludge amended soil accumulated cadmium. Glutathione content of both young and old leaves was significantly higher in the sludge-grown vegetables than in the corresponding control plants, likely in response to stress factors imposed by the sludge amendment. Putrescine, spermidine, and spermine, measured only in the collards, also increased, possibly by induction due to cadmium accumulation or to general metal stress. Cabbage loopers foraging on the sludge-grown plants assimilated and concentrated cadmium from the contaminated foliage. Putrescine, spermidine, and spermine also increased in the loopers associated with the collards whereas glutathione did not. The insects grew larger but developed more slowly on the sludge-grown collards than on the corresponding control plants. Possible accumulation of cadmium in insects by insect-consuming birds is discussed.

A single municipal wastewater treatment facility may treat the effluents of several hundred industries as well as thousands of domestic users. Therefore, the resulting sewage sludges may typically contain a broad spectrum of organic and inorganic toxicants and other constituents of largely industrial origin (Furr et al., 1976a; Babish et al., 1981; Mumma et al., 1983, 1984). When sludge is applied to land, the trace element of most concern is cadmium since it is toxic, readily absorbed by plants (Furr et al., 1976b), and concentrated in tissues of foraging animals (Babish et al., 1984). Owing to the galaxy of organic and inorganic toxicants that may be present in municipal sewage (Babish et al., 1981), the possibility of development of plant stress from growth in such an unnatural medium as sludge-amended soil is of interest. Furthermore, no research has been reported on the transfer of toxicants from sludge-grown plants to insects feeding on such plants and possible concomitant stress effects in such plants and insects.

Increases in the concentration of the diamine putrescine (Flores et al., 1984; Galston, 1983; Smith, 1973, 1984, 1985), and glutathione (Hughes et al., 1985a; Chiment et al., 1986) in plants have been reported to serve as possible indicators of various forms of plant stress. In the work reported here, the concentration of these indicator compounds and cadmium were determined in potted cabbage and collard plants grown on municipal sludge amended soil and in cabbage loopers (*Trichoplusia ni*) with which these plants were deliberately infested.

METHODS

The sludge used was obtained from the Ley Creek wastewater treatment plant in Syracuse, NY. This facility receives the effluents discharged by about 100 industries as well as domestic wastes. The facility produces an anaerobically digested, waste-activated sludge. Table I lists concentrations of macro and micro plant nutrient elements and trace metals in the sludge. The pH of the sludge was 7.1, and its ash content was 48.4%. In an analytical survey of the elemental content of 30 municipal sludges collected in the United States in 1980, the cadmium content ranged from 3.3 to 203 ppm (Mumma et al., 1984). On that basis, the sludge used in this study containing 76 ppm cadmium (Table I) would be classified as intermediate in cadmium content. The soil was an Eel silt loam (fine-loamy, mixed, nonacid, mesic Aquic Udifluvents), pH 6.82. The soil and sludge were air-dried and sifted through a 2-mm screen. Ten percent (w/w) of the sludge was thoroughly mixed with the soil (100 dry tons of sludge/acre or 224 metric tons/ha) in a cement mixer. This rate of sludge application would simulate that which accumulates on dedicated land sites. Considering the initial soil cadmium content (Table II) and that added with the sludge, the soil–sludge mixture therefore contained 8.9 ppm. The pH of the final sludge–soil mixture was 6.88. The sludge–soil mixture was placed in 36 plastic pots, 22.9-cm diameter and 21.6-cm height, each containing 7125 g of the mixture and having drainage holes. Controls consisted of 36 similar pots

Table I. Elemental Composition of the Sludge

constituent	%	ppm
Ca	2.9	
Fe	0.7	
K	0.02	
Mg	0.6	
N	2.0	
Na	0.3	
P	1.9	
B		21.4
Cd		76.0
Co		8.6
Cu		1860
Mn		135
Ni		390
Pb		372
Zn		5780

Table II. Concentrations of Cadmium in Cabbage and Collard Leaves, the Associated Loopers, and Soil

sample[a]	Cd (ppm dry wt) control	Cd (ppm dry wt) sludge
cabbage leaves	0.39	4.89
loopers on cabbage, prepupae	0.24	1.80
collard leaves	0.11	2.40
loopers on collards, adult male	0.80	3.92
adult female	0.79	2.88
soil	1.40	

[a] Leaf and insect samples were a composite of those taken from each pot.

Boyce Thompson Institute for Plant Research (P.R.H., L.H.W., S.H.W., J.J.C.), Departments of Vegetable Crops (G.J.D.) and Entomology (T.W.C.), and Toxic Chemicals Laboratory (W.H.G., C.A.B., D.J.L.), New York State College of Agriculture and Life Sciences, Cornell University, Ithaca, New York 14853.

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containing the same weight of soil alone. To each pot of soil or sludge–soil was added 21.5 g of 18-7-10 (N–P_2O_5–K_2O) controlled release fertilizer (Sierrablend Nursery Mix, Sierra Chemical Co., Milpitas, CA), which also contained 1% iron.

Cabbage Test. One Golden Acre cabbage (*Brassica oleracea* var. Capitata) seedling, 14 days old, was then transplanted into each of 18 control and 18 sludge-containing pots. The plants were watered daily, and each was fertilized weekly for the first 2 weeks with 700 mL of a solution containing 540, 576, and 612 ppm, respectively, of N, P and K. The forms of nitrogen in the fertilizer were nitrate (53.3%), ammonium (21.2%), and urea (25.5%). The plants were grown in a greenhouse at 27 ± 1 °C. Eighty days after transplanting, when cabbage heads had begun to form, 24 second-instar cabbage loopers from a laboratory colony (Hughes and Wood, 1981) were placed on each of the plants. The loopers were removed from the leaves as they pupated and were freeze-dried in preparation for analysis. Young cabbage leaves were sampled from each pot in a given treatment and combined for determination of cadmium. Soil and sludge samples were also taken for analysis. Tissue from both young and old leaves was weighed and placed in liquid nitrogen for analysis of glutathione.

Collard Test. One "Vates" collard (*Brassica oleracea* var. Acephala) seedling, 14 days old, was transplanted into each of 18 control and 18 sludge-containing pots. The plants were placed in the greenhouse under the above conditions until 10 days after transplanting, at which time they were transferred to a growth room with the following conditions: temperature, 21 ± 0.5 °C (day), 18 ± 0.5 °C (night); relative humidity, 65–75% (day), 90–100% (night); 16:8 light:dark cycle with an abrupt change; light intensity, 900 ± 100 μeinstein/m^2 per s (400–700 nm). Internal volume of the growth room was 61 m^3, and fresh, filtered makeup air was provided at the rate of 2 m^3 min^{-1}. Lighting was provided by equal numbers of 400-W multivapor, high-intensity discharge lamps (General Electric MVR400/IU, 12OV) and Lucalox 400-W high-pressure sodium lamps (General Electric LU400, 120V). Temperature and humidity were measured by means of a shaded Model 207 temperature/humidity probe (Campbell Scientific, Inc., Logan, UT) located approximately 15 cm above the plant tops. Light intensity was measured by a Licor LI190SB quantum sensor (Li-Cor, Inc., Lincoln, NE) at the same height. The probe and sensor were connected to a Model 21X Micrologger (Campbell Scientific, Inc.) for continuous monitoring. In addition, recordings from three hygrothermographs placed around the light room showed that temperature and humidity were very uniform throughout the test area. For the last 8 days during which pupation was occurring, the temperature of one senescing (yellow) and one mature leaf from each treatment was monitored by 0.025-mm chromel-alumel thermocouples pressed against the lower leaf surfaces and connected to the datalogger. Air flow, as measured with a Datametrics 100VT hot-wire anemometer, was predominantly downward with a velocity of approximately 15 m/min at the tops of the plants.

The plants were watered automatically twice daily with deionized water with the amount adjusted and supplemented by hand watering as needed to keep the pots moist with a minimum of runoff. When the plants were 65 days old, 20 newly hatched cabbage looper larvae were placed on each plant. As they pupated, the loopers were removed from the leaves, weighed, and placed individually in 1-oz plastic cups. They were returned to the light room and checked daily to determine time to adulthood, and the sex of each individual was recorded as adults emerged.

When all of the insects had been removed from the plants, leaves were taken from each plant for various analyses. Since sufficient material to accomplish all of the desired analyses could not be obtained from a single leaf, specific nodes were designated for given analyses. The oldest nonsenescing leaf (L1) was taken for nitrogen and mineral analysis. Leaves two to three nodes above L1 were used for cadmium analysis, and those five nodes above (old leaves) were used for glutathione analysis. The youngest fully unrolled leaf (new leaf) was used partly for glutathione analysis and partly for polyamine analysis. After the latter leaves were removed for analyses, the remaining leaves were stripped from the plants and fresh and dry weights of the stems determined in order to provide a comparison of relative plant growth between the treatments.

Leaf and Insect Analyses. Leaf and insect samples for cadmium analyses were frozen and lyophilized immediately. For analysis, the samples were wet-ashed with nitric, sulfuric, and perchloric acids. Cadmium was determined in the acid digests by conventional stripping voltammetry (Gajan and Larry, 1972).

Samples for glutathione analyses were immediately weighed and placed in liquid nitrogen. Glutathione was estimated as total water-soluble sulfhydryl by the method of Sedlak and Lindsay (1968) as modified by Chiment et al. (1986).

Leaf samples for nitrogen and foliar analyses were oven-dried at 90 °C for 48 h, ground through a 40-mesh screen by means of a Wiley mill, and stored in a desiccator until analyzed. Multielement analyses of the tissue were accomplished by inductively coupled argon plasma atomic emission spectroscopy (Dahlquist and Knoll, 1978).

For analysis of polyamines, the samples were homogenized in cold 10% perchloric acid at a ratio of 100 mg/mL. The supernatant fraction was collected after centrifugation at 12000g for 20 min. Dansyl derivatives of the polyamines were then prepared, separated by thin-layer chromatography, and analyzed as described by Flores and Galston (1982).

Eight collard plants were randomly chosen from each treatment for analysis of leaves and associated loopers. Glutathione analyses were conducted for all of the plants while cadmium, nitrogen, and polyamine analyses were conducted for six of the eight plants from each treatment. Mineral analyses were made of two of these latter six plants. Insects had to be pooled for analysis, yielding sufficient material for four, three, and two samples per treatment for glutathione, polyamine, and cadmium analyses, respectively.

Statistical Analyses. Statistical treatment of results included analysis of variance and Student's t-test (Steel and Torrie, 1960) or the Mann-Whitney nonparametric rank test (Hollander and Wolfe, 1973).

RESULTS AND DISCUSSION

Cadmium was accumulated to significantly higher concentrations by leaves of both cabbage or collards (Table II) when grown in the sludge–soil mixture ($p = 0.001$, two-tailed Student's t-test). Furthermore, looper larvae feeding on the contaminated leaves accumulated significant amounts of the metal ($p = 0.037$, two-tailed Student's t-test).

Concentrations of the di- and polyamines putrescine, spermidine, and spermine in the collards and associated loopers from randomly chosen pots are given in Table III. Each of the amines was significantly higher ($p < 0.05$) in

Table III. Concentrations of Putrescine, Spermidine, and Spermine in Collard Leaves and the Associated Loopers

treatment	no. of pots[a]	putrescine,[b] nM/g fresh wt	no. of pots	spermidine, nM/g fresh wt	no. of pots	spermine, nM/g fresh wt
control leaves	4	8.6 ± 5.9[x]	8	83.4 ± 20[x]	8	70.5 ± 7.4[x]
sludge leaves	8	28.6 ± 9.7[y]	7	186 ± 18[y]	8	91.6 ± 5.1[y]
control loopers	3	1449 ± 80[x]	3	597 ± 22[x]	3	148 ± 5.5[x]
sludge loopers	3	2314 ± 12[y]	3	800 ± 6.1[y]	3	198 ± 18[y]

[a] Number of pots from which leaves or loopers were sampled. [b] Mean ± standard error; dissimilar letter superscripts indicate significant differences ($p < 0.05$) between treatment means and their respective controls. Statistical analyses for leaf samples were made by the Mann–Whitney nonparametric rank test.

Table IV. Concentrations of Glutathione in Cabbage Leaves, Collard Leaves, and (Collard) Loopers

treatment	no. of pots[a]	glutathione,[b,c] nM/mg
control cabbage (new leaves)	8	0.28 ± 0.01[x]
sludge cabbage (new leaves)	8	0.40 ± 0.02[y]
control cabbage (old leaves)	8	0.42 ± 0.02[x]
sludge cabbage (old leaves)	8	0.57 ± 0.02[y]
control collards (new leaves)	8	0.33 ± 0.03[x]
sludge collards (new leaves)	8	0.53 ± 0.03[y]
control collards (old leaves)	8	0.26 ± 0.03[x]
sludge collards (old leaves)	8	0.38 ± 0.03[y]
control (collard) loopers	4	4.24 ± 0.34[x]
sludge (collard) loopers	4	4.05 ± 0.43[x]

[a] Number of pots from which leaves or loopers were sampled. [b] Nanomoles of water-soluble nonprotein-SH/milligrams (fresh weight) of leaf or insect tissue. [c] Mean ± standard error; dissimilar letter superscripts indicate significant differences ($p < 0.05$) between treatment means and their respective controls.

both leaves from sludge-grown plants and insects that had fed upon these plants. Weinstein et al. (1986) have shown that putrescine increases severalfold in detached bean or oat leaves from intact plants treated with solutions containing cadmium. Little effect was seen, however, on levels of spermidine or spermine. While cadmium was the only stress imposed in their studies, plants grown in sludge-amended soil may have been exposed to a number of potentially phytotoxic chemicals, since sludge may contain a wide range of metals and synthetic compounds. Also, the observed induction of polyamine synthesis in our tests cannot be unequivocally associated with cadmium uptake alone, since many stresses are known to stimulate production. For example, leaves from sludge-grown plants had only about half the potassium of the controls and Smith (1973, 1984) has shown striking accumulation of putrescine in potassium-deficient plants. Whether the increased levels of amines in the looper larvae were due to induction in the insects by constituents from the sludge-grown plants or from assimilation of endogeneous polyamines from the ingested plant tissue is unknown at present. Strong induction by ingested cadmium of ornithine decarboxylase, the enzyme responsible for polyamine biosynthesis in animals, was recently reported in rat renal and hepatic tissues (Yoshida et al., 1986), and the possibility of a similar phenomenon in insects is worth investigating.

Total amounts of reduced glutathione in cabbage leaves, collard leaves, and loopers reared on the collards are listed in Table IV. Concentrations in both young and old leaves of sludge-grown plants were significantly higher ($p < 0.05$) than the respective controls. Glutathione levels did not differ significantly between loopers fed control or treated plants. Recently, Grill et al. (1985a, 1985b) discovered a novel set of peptides whose synthesis in cultures of many different plant cells was induced by cadmium. These compounds, called phytochelatins, are structurally related to glutathione and contain many free sulfhydryl groups. Therefore, we cannot discount that some or all of the increase we considered to be glutathione may actually be phytochelatin.

By 65 days after planting, the collard plants grown in the sludge–soil mixture were noticeably greener and larger than those grown in soil alone. This difference was reflected in significantly greater mean fresh and dry weights of the stems at harvest (33.47 vs. 16.67 g fresh weight and 7.84 vs. 5.05 g dry weight, for sludge-grown and control plants, respectively; $t = 8.7507$ with 26 degrees of freedom). The percent moisture of stems from sludge-grown plants was also significantly greater than that of controls (76.5% and 69.7%, respectively; $t = 9.159$ with 26 degrees of freedom, Student's t-test on angularly transformed data). Foliar analyses of leaves from these plants showed that both treatments contained concentrations of Ca, P, Na, and K comparable to literature values for collards (Watt and Merrill, 1975). The sludge-grown plants were significantly higher than the controls in each of the elements listed in Table V with the exception of Mn, Na, and Co. Plant nutrient elements such as N and P have been reported to increase in other studies in which crops were grown on sewage sludge amended soils (Stark and Clapp, 1980; Magdoff and Amadon, 1980; Logan and Miller, 1985).

Temperature of mature leaves in direct light with laminae oriented perpendicularly to the source was generally 1–1.5 °C above ambient during the day and 0.4 °C below ambient at night. Senescent leaves similarly positioned ranged from 2 to 8 °C above ambient, generally averaging about 4 °C higher. The mature control leaf was approximately 1 °C warmer during the photophase than the corresponding leaf on the sludge-grown plant throughout the 8 days of monitoring.

Both male and female loopers reared on sludge-grown collards grew larger and developed more slowly than those reared on the control plants, but the difference for these two characteristics was only significant in the case of the males (Table VI). While these differences were not sta-

Table V. Mineral Content of Leaves from 100-Day-Old Collard Plants Grown in Sludge-Amended Soil (Treated) or Soil Alone (Control)

	mineral[a]										
treatment	N	P	K	Ca	Mg	Mn	Cu	Zn	Na	Co	Ni
control	0.92	0.17	1.06	1.91	0.20	36.8	1.97	10.71	497	1.05	1.59
sludge	1.20	0.31	0.59	2.74	0.33	50.0	7.48	149.08	613	1.54	4.20
P value[b]	0.03	0.004	0.01	0.003	0.03	0.11	0.01	0.01	0.55	0.44	0.01

[a] Values are in percent dry weight for N, P, K, Ca, and Mg and parts per million for the remaining elements and are means of analysis of replicates ($n = 6$ for N and 4 for each of the other elements). [b] Data were analyzed by Student's t-test.

Table VI. Pupal Weight and Time to Adulthood of Cabbage Loopers Reared on Collards Grown in Soil Amended with Sewage Sludge or Soil Alone

treatment	sex	no. of loopers[a]	wt,[b] mg	no. of loopers	time to adult,[b] days
control	M	42	179 ± 3.6[x]	36	29.5 ± 0.5[x]
sludge	M	44	184 ± 3.0[y]	42	31.4 ± 0.9[y]
control	F	40	193 ± 3.7[x]	35	30.5 ± 0.5[x]
sludge	F	35	209 ± 3.8[x]	32	33.0 ± 0.9[x]

[a] Number of pots from which leaves or loopers were sampled. [b] Mean ± standard error; dissimilar letter superscripts indicate significant differences ($p < 0.05$) between treatment means and their respective controls.

tistically significant for females ($p < 0.27$ and 0.059 for growth and development times, respectively), male loopers showed significantly higher pupal weights ($p < 0.004$) and longer times to reach adulthood ($p < 0.02$). The difference in rate of development (1.9 days for males, 2.5 days for females) is within the range of what might be produced by the difference in leaf temperature. For example, from development data from Hughes et al. (1985b) for *T. ni* on cabbage, a change in daytime temperature from 21 to 25 °C would be expected to decrease development time by approximately 2 days. Alteration of growth, rate of development, and fecundity of insects by environmentally induced changes in their host plants is well documented (White, 1984), and the success of at least one insect is increased by elevation of foliar glutathione (Hughes and Chiment, 1985). Although such an effect cannot be discounted in these studies, the decreased growth, lower foliar nitrogen, and higher leaf temperatures of the control plants suggest that differences in plant nutrition and leaf temperature may have caused the observed decreased growth and increased rate of development of insects on these plants.

Relatively few studies have been conducted of the effects of sewage constituents on invertebrates. In aquatic ecosystems, both increases in the populations of lake nematodes (Smith, 1979) and decreases in those of dragonflies (Watson et al., 1982) have been reported. Sediments with high concentrations of cadmium and other metals have also been shown to severely reduce growth and population densities of midge larvae (*Chironomus tentans*) (Wentsel et al., 1977). Howell's (1984) findings indicated that marine nematodes from a polluted site were more tolerant of elevated levels of metals. Ingersoll and Winner (1982) found that *Daphnia pulex* (deGeer) showed no observable toxic effects if exposed to pulse concentrations of cadmium in water such that the daily average concentration did not exceed the chronic, no-observable-effect concentration. Other studies of the effects of metal contamination on population shifts (Zanella, 1982) and toxicity (Warnick and Bell, 1969) to aquatic insects have been published. Soil invertebrates also could be exposed directly to sludge particles or vapors from freshly applied sludge. In this regard, metals such as cadmium have been shown to be greatly concentrated by earthworms inhabiting municipal sludge amended soils (Helmke et al., 1979; Wade et al., 1982). Since the size and rate of male and female looper development was quite comparable between the sludge-grown and control collards in this study, it would indicate that the concentrations of metals present in the plant tissue did not inhibit their feeding habits or cause toxicity.

Plant-feeding insects could presumably consume and be affected by any constituents present in sludge that are absorbed by plants, but no direct studies of this possibility were found in the literature. Polek and Weismann (1984) found that 95.2% of the cadmium fed to caterpillars (*Scotia segetum*) was contained in the digestive tract, with only 4.8% passing through the gut wall to be deposited in other tissues. Sumi et al. (1984) reported similar findings in midge (*Chironus yoshimatsui*) larvae fed a cadmium-containing diet. Suzuki et al. (1984) found 84% of the cadmium accumulated by larvae of the silkworm (*Bombyx mori*) fed a contaminated artificial diet was located in the alimentary canal, 11% in the Malpighian tubules, and the remainder throughout various other tissues. Wade et al. (1982) reported that 69.8% of the total body burden of cadmium in earthworms inhabiting sewage sludge amended soil is contained in their gut contents. If deposition and excretion of cadmium are similar in cabbage loopers, gut contents at the time of insect sampling for cadmium analysis could be a major factor affecting the concentration found.

None of the above studies investigated toxicity of cadmium, but Martoja et al. (1983) found various toxic effects of it in the locust (*Locusta migratoria*), with the toxicity depending on age and sex of the insects. It is interesting that a cadmium-binding complex in cabbage leaves has been reported (Wagner, 1984) and that accumulation of cadmium in the digestive tract and induction of a cadmium-binding protein was found in fleshfly (*Sarcophaga peregrina*) larvae fed a cadmium-containing diet (Aoki et al., 1984). The protein was a mixture of five isoproteins with several properties characteristic of metallothionein. Similar results were reported by Suzuki et al. (1984) in the silkworm.

Our studies indicate that at least one generalist feeder, the cabbage looper, is not very sensitive to cadmium or certain other metals (Table V) and possibly organic compounds absorbed by two of its plant hosts from the sludge. In spite of elevated cadmium concentrations in the cabbage loopers, no obvious toxic effects on growth or survival of this insect were observed. Possibly relevant to these results, Medici and Taylor (1967) reported a protective effect of zinc against cadmium toxicity in the confused flour beetle (*Tribolium confusum*) and the zinc content of the sludge-grown collard plants in our experiment was quite high (Table V).

Due to its toxicity, biomagnification of cadmium through trophic levels is of great concern. Pimentel et al. (1984) have shown considerable accumulation of cadmium in the tissues of Japanese quail fed high cadmium-containing earthworms collected from a golf course that had received annual applications of municipal sewage sludge and cadmium-containing fungicides. Also, high levels of cadmium have been found in marine insects (Cheng et al., 1976, 1979) and in their seabird predators (Cheng et al., 1984). Obviously, insect-consuming birds or fish could accumulate high concentrations of cadmium from their prey. Our results indicate that this would likely be important in predators of phytophagous insects as well.

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Registry No. Cd, 7440-43-9; N_2, 7727-37-9; P, 7723-14-0; K, 7440-09-7; Ca, 7440-70-2; Mg, 7439-95-4; Mn, 7439-96-5; Cu, 7440-50-8; Zn, 7440-66-6; Na, 7440-23-5; Co, 7440-48-4; Ni,

7440-02-0; putrescine, 110-60-1; spermidine, 124-20-9; spermine, 71-44-3; glutathione, 70-18-8.

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