Mineral Content of Selected Tissues and Feces of Rats Fed Turnip Greens Grown on Soil Treated with Sewage Sludge

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Weanling rats were fed dried leaf and stem material from turnip greens grown on soils treated with relatively high rates of industrial type, secondary treated sewage sludge or with inorganic fertilizers. Diets contained 50% plant material and adequate quantities of essential organic nutrients. A standard diet without plant material was fed to one group of rats also. Samples were analyzed for Ca, P, K, Mg, Mn, Fe, Zn, Cu, Cd, Cr, Ni, and Pb. Feed consumption was reduced by inclusion of dried greens in the rat diets and digestibility of the greens (as indicated by feed efficiency and dry weight of feces excreted) was adversely affected by sludge treatment. Evaluation of the determined elements showed that only liver and kidney tissue cadmium appeared to be influenced by diets derived from turnip greens produced on soil amended with industrial type sewage sludge. The cadmium content of these tissues was higher in rats fed the greens grown on sludge-treated soil than in rats fed greens from the plot treated with NPK fertilizer or in those fed the standard diet.

Application of sewage sludge to agricultural land offers both a source of essential nutrients for crop production and means of disposal. The possibility that sewage sludge from industrial areas may contain levels of heavy metal that are toxic to plants is well recognized (Allaway, 1968; Page, 1974). The potential transfer of elements through plants along the food chain to meat animals and man at levels that might be toxic is of great concern also. Although there are numerous studies of effects of sludge-borne heavy metals on plant composition, there are few reports concerning the ultimate effects on animals consuming crops grown on land treated with sewage sludges with high heavy metal content.

In a previous study, we reported that grain of corn, sorghum, and soybean grown on sludge-treated land had little effect on mineral content of liver and kidney of rats fed the grain at levels of 80% of nutritionally adequate diets (Miller and Boswell, 1976). When winter wheat was grown on fly ash that had a high concentration of selenium, the metal was taken up by the plant and transferred to tissues and eggs of quail fed the grain (Stoewsand et al., 1978). In the above studies, the animals were fed the fruit portion of the plants. Generally, leaves will accumulate more heavy metals than will the fruits (Kirkham, 1974). Furr et al. (1976) fed Swiss chard grown in sludge-amended soil to guinea pigs. They noted excessive accumulations of antimony, cadmium, manganese, and tin in some animal tissues but observed no toxicological effects on the guinea pigs.

In the experiment reported here, turnips were grown on soil treated with municipal sewage sludge and fed as 50% of the diet to weanling rats. In addition to measuring content of several elements in selected animal tissues, the proportion of the dietary intake that was excreted in the feces was also assessed.

MATERIALS AND METHODS

Three blocks (12.2 × 15.2 m each) were established in the field on a Cecil sandy loam soil (clayey, kaolinitic, thermic Family of Typic Hapludults) that had an initial pH of 5.6. One block (NPK, inorganic nitrogen, phosphorus, and potassium) received 168 kg/ha of nitrogen as ammonium nitrate and 134 kg/ha of potassium as potassium chloride in the fall of 1976. The other two blocks received 11.2 (LSS, low sewage sludge) and 22.4 (HSS, high sewage sludge) mton/ha (dry weight basis), respectively, of industrial type (Atlanta, GA), secondary digested sewage sludge. Composition of the sludge (Knezek and Miller, 1978) and rate of application to the LSS block of selected minerals contained therein are shown in Table I. Purple top, white globe turnips were seeded to a depth of 7–10 cm after incorporation of sludge and fertilizers. Due to an early freeze, only a few seedlings survived and the test site was temporarily abandoned and fallowed during the spring and summer of 1977. The above described materials were again applied in the fall of 1977, except 672 kg/ha of 5-10-15 complete fertilizer was applied with additional ammonium nitrate to result in 168 kg/ha of nitrogen on the NPK block. Turnips were seeded at the rate of 160 g/100 m^2 and disked into the surface 4–5 cm. After a vigorous 8-week growth period in October and November, above-ground whole plants were removed, washed three times with deionized water, and dried at 70 °C for 120 h. Tissue was ground with a Wiley mill to pass a 50-mesh screen and stored in plastic bags for subsequent diet mixtures.

Table I. Chemical Content of Sewage Sludge and Rates of Elements Applied

element	content, ppm	total applied,[a] kg/ha
N	19 700	441
P	16 800	377
K	1 500	34
Ca	17 400	389
Mg	2 240	50
Mn	949	21
Fe	24 900	558
Zn	7 590	170
Cu	512	12
Cr	1 040	23
Cd	114	3
Pb	2 560	57
Ni	189	4

[a] Total amount applied to LSS plot in 1976 and 1977; HSS plot received twice as much sludge as LSS plot.

The dried plant material was incorporated as 50% of the total weight of rat diets (Table II). Casein (supplemented with methionine) and a complete vitamin mixture were added at the same concentration as that used in the standard diet so that essential organic nutrients were pro-

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Table II. Ingredients of Diets (g/kg)

ingredients	standard	exptl
casein	156.5	156.5
l-methionine	2.0	2.0
vitamin mixture[a]	22.0	22.0
soybean oil	200.0	200.0
turnip greens		500.0
cellulose	150.0	
corn starch	217.2	
sucrose	217.2	97.5
salt mixture[b]	35.0	
$CaHPO_4$		12.0
Na_2HPO_4		9.0
NaCl (iodized)		1.0

[a] Formulated according to Vitamin Diet Fortification Mixture, ICN Nutritional Biochemicals Corporation.
[b] Mix UCB-1Rb (Cohen et al., 1967).

Table III. Composition of Turnip Greens,[a] Dry Weight Basis

	soil treatment		
	NPK	LSS	HSS
ADF,[b] g/kg	230	210	240
total ash, g/kg	128	156	177
nitrogen, g/kg	42.5	36.0	35.1
calcium, g/kg	13.4	22.5	27.9
phosphorus, g/kg	7.5	7.5	6.9
potassium, g/kg	41.9	46.5	48.0
magnesium, g/kg	2.1	3.7	4.2
manganese, mg/kg	280	106	66
iron, mg/kg	377	526	679
zinc, mg/kg	83	346	421
copper, mg/kg	7.7	8.6	9.4
cadmium, mg/kg	1.0	3.6	4.4
chromium, mg/kg	1.7	2.2	2.5
lead, mg/kg	7.8	10.5	12.3
nickel, mg/kg	3.0	7.5	9.4

[a] Turnip greens grown on soils treated with conventional fertilizers (NPK), low sewage sludge (LSS), and high sewage sludge (HSS) rates. [b] ADF, acid detergent fiber.

vided at required levels without reliance upon those contained in the dried turnip greens. Soybean oil provided essential fatty acids and was added at 20% of the diets to compensate for the nondigestible carbohydrate and ash of the plant material (Table III) by increasing the caloric density of the diet. Dibasic calcium phosphate was added to provide some of the essential bone building minerals in biologically available form. Sodium phosphate was included to increase the ratio of phosphorus to calcium. Sucrose provided readily available carbohydrate and increased palatability of the diet. The standard diet provided adequate amounts of all known nutrients to support rapid growth of weanling rats. A relatively high level of cellulose (15%) was used to approximate content of the nondigestible material in the turnip greens.

Weanling rats were maintained on the standard diet for the initial 48 h after arriving in the laboratory and then sorted into eight groups of eight animals each on the basis of body weight. Two of the groups were fed a standard diet (Table II) and two were fed the diet containing each lot of turnip greens for 28 days. The rats were housed individually in stainless steel cages with wire mesh floors and provided with diet and deionized water ad libitum. Feces were collected each morning during the feeding period from one group of rats fed each diet and stored at −5 °C for subsequent analysis. Feed intake of rats in these groups was also monitored, with any food spilled being picked up each morning and subtracted from intake.

At the end of the feeding period, rats in these four groups were given a lethal dose of pentobarbital intraperitoneally. After death of the rat, food particles and other debris were removed from the fur by vacuuming. The peritoneal cavity was opened and the GI tract and its contents, from the proximal end of the esophagus to the anus, was removed, taking care that no blood loss occurred. The remaining part of the rat, hereafter referred to as "carcass", was disintegrated by digestion in nitric acid. The fat, which rose to the top of the digestion vessel after cooling, was discarded and the aqueous portion was made to volume from which aliquants were taken for mineral analysis.

Rats of the second group fed each diet were anesthetized and blood from the heart was collected into heparinized syringes for preparation of serum by centrifugation. After complete exsanguination, the liver, kidneys, heart, and testes were removed and freeze-dried for subsequent analyses.

Aliquants of serum and the carcass digest, weighed portions of the dried greens, diets, and liver, and the whole tissue of other organs were digested in nitric and perchloric acids. Inorganic elements were determined by atomic absorption spectroscopy, utilizing a deuterium background corrector when applicable. Kjeldahl nitrogen and acid detergent fiber (ADF) (Van Soest, 1963) were determined on dried greens samples.

Data were subjected analyses of variance and differences among means evaluated by Duncan's multiple range test where appropriate (Duncan, 1955).

RESULTS AND DISCUSSION

Yields of plant material were not quantitatively evaluated but visual observations indicated that greatest production occurred with the HSS rate while the LSS rate was intermediate and inorganic fertilizers resulted in the least yield. No obvious adverse effects of heavy metals from the sewage sludge were noted in the turnip greens during growth. Greens grown on the plots treated with the sludge were higher in ash content than were those produced on the NPK plots, and ash content was greater in plants from the HSS plot than in those from the LSS plot (Table III). Calcium content of the plants was increased markedly by sludge treatment of the soil. Iron and, especially, zinc were taken up in great amounts by turnips grown in the presence of the sewage sludge. Cadmium and nickel concentrations in the plant material were increased several fold by application of the sewage sludge, while potassium, magnesium, copper, chromium and lead contents were increased to lesser extents. The turnip plants took up more manganese from the plot treated with NPK fertilizer than from plots to which sludge was applied. While the difference in uptake may have been due in part to the chemical form of manganese in the soil, it was probably influenced also by soil pH. The pH of the plot treated with NPK was lower (5.2) than that of the sludge-treated plots and the LSS plots had a lower pH (5.6) than the HSS block (5.8). Manganese uptake into plants is favored by an acid soil pH. Thus, treatment of the soil on which they were grown with sewage sludge had both direct and indirect (e.g., Mn) effects on composition of turnip greens but no apparent adverse effects on yield of total dry plant tissue.

In addition to the above evaluations, a major objective of this study was to compare effects on growth and tissue mineral content of rats fed diets containing turnip greens grown on sludge-treated soil with those containing plant material produced on soils treated with conventional fertilizers. Animals fed standard diets without dried tissue were included for comparison and to identify effects due to dietary additions of plant tissue, per se. However, no source of minerals other than the dried plant material was

Table IV. Growth Performance[a] of Rats Fed a Standard Diet and Diets Containing 50% Dried Turnip Green Tissue[b]

constituent	standard	NPK	LSS	HSS
food intake, g	444a	372b	370b	404ab
feed efficiency[c]	0.459a	0.458a	0.430b	0.406c
final weight, g	267a	229b	218b	222b
liver weight, g	12.1a	13.6a	13.5a	12.7a
% of body wt	4.5b	5.9a	5.9a	5.9a
kidney weight, g	2.2a	2.5a	2.3a	2.2a
% of body wt	0.8b	1.1a	1.0a	1.0a
heart weight, mg	960a	761a	807a	744a
feces dry weight, g	73c	88b	92b	115a

[a] In each row, values having no common letter are significantly different at $P \leqslant 0.05$. [b] Turnip greens grown on soils treated with conventional fertilizers (NPK), low sewage sludge (LSS), and high sewage sludge (HSS) rates. [c] Feed efficiency is calculated as weight gained divided by feed intake for the 28-day feeding period.

deliberately added to the experimental diets except for calcium phosphate, sodium phosphate, and iodized table salt. Thus, the well being of the animals was dependent upon biological availability of nutritionally essential mineral elements from the turnip greens.

Analyses after all ingredients were added indicated that the diets contained higher chromium, lead, and nickel levels than could be accounted for by the amount of these elements contributed by the turnip greens. The experimental diets as fed to the rats contained about 6, 9, and 13 mg/kg of chromium, lead, and nickel, respectively. The contamination probably came from the stainless steel equipment in which the diets were mixed.

Dried turnip greens, included as 50% of the total weight of the diets, resulted in decreased food intake, decreased growth, and increased size of liver and kidneys relative to body weight when compared to performance of rats fed the standard diet (Table IV). Since these changes occurred with greens grown on plots treated with inorganic fertilizer as well as with the ones from sludge-treated soil, they were likely due to effects of turnip greens, per se, rather than to type of land treatment. The reduction in food intake by rats fed diets with the greens may have been caused by either a platability factor or some physiological action of the greens and would have contributed to their lower weight gain. However, the increase in dry weight of feces excreted indicates that the greens were also less digestible than the replaced carbohydrates in the standard diet.

The reduced feed efficiency of rats fed turnip greens produced on the LSS and HSS plots compared to that of the animals fed diets containing turnip greens from plots treated with NKP fertilizer indicates that sludge has an adverse effect on nutritional quality of the turnip greens. Furthermore, the lower feed efficiency and greater feces excretion by rats fed HSS greens compared with those fed LSS greens suggest that effects are proportional to the amount of sludge applied.

Utilization of the major minerals, calcium, phosphorus, potassium, and magnesium, from these diets (Table V) demonstrates the close homeostatic control that mammalian systems have over concentration of these elements within the body. Though the four diets presented the organisms with major differences in intake of the minerals, the differences in amounts accumulated in the carcasses were related to total body weight.

Thus, for each of the four minerals the carcass content was less in rats fed the diets containing the dried turnip greens than in those fed the standard diet, and these differences were proportional to differences in weight of the rats at the end of the feeding period.

Much of the excess calcium in the diets containing turnip greens was excreted in the feces. There was also a large excess of phosphorus in the feces of rats fed these diets. This may indicate that some of the calcium and phosphorus in the leafy material was present in a form not solubilized by the digestive process. The greens supplied very high levels of potassium to the diets, much of which was absorbed into the body rather than being excreted into the feces. Livers of the rats fed the diets with greens grown on NPK and LSS plots had more potassium than those of rats given the standard diet or the diet containing the leaves produced with the high level of sludge. Much of the excess magnesium supplied by the turnip greens was excreted in the feces but the amount absorbed also increased with increasing dietary supply by greens grown on the NPK, LSS, and HSS plots, respectively. Most of the excess of magnesium that was absorbed must have been excreted in the urine, however, for there was no excessive accumulation in the carcass or in any of the tissues examined.

Most of the manganese consumed by the rats was excreted in the feces, especially when the mineral was supplied by turnip greens rather than by the inorganic salt in the standard diet. The particularly high rate of fecal excretion of manganese derived from the leafy material produced with the NPK fertilizer indicates that the manganese of this material was essentially unavailable to the rat. The sludge-treated plots, on the other hand, yielded greens that did supply the rats with some metabolically available manganese. The amount of manganese in the liver of rats fed the dried plant material was greater than that in liver of rats fed the standard diet. The effect appeared to be due to the greens rather than to the source of manganese, as content of manganese in the livers of rats fed greens from LSS and HSS plots was not significantly different from that of rats fed greens from the NPK treated plot.

The turnip greens contributed large quantities of iron to the diets of the rats. Iron content of the sewage sludge was about 2.5% (dry weight) and the turnip leaves accumulated large quantities of this mineral. Most of the iron supplied by the dried greens was excreted in the feces and, as a result, total iron in the carcass was not significantly different among the dietary treatments, despite the great differences in intake. The amount of iron in the livers of rats fed the diets containing the leafy material, especially that produced on the NPK and LSS plots, was greater than that of the rats given the standard diet. The lower liver iron content of rats fed the greens from the HSS plots, compared to that of animals fed the other two lots of greens, suggests that the sludge-derived iron of the greens may have been less available to the rats than from other sources. The source of dietary greens had no apparent effect on the iron content of kidney, heart, or testes.

Sludge treatment had greater effect on increasing the content of zinc in the plant tissue than on any other constituent measured. Zinc concentration in the dried greens grown on the LSS and HSS plots was four and five times, respectively, that of the plants from the plot treated with NPK fertilizer. Most of the zinc from turnip green tissue was excreted in the feces of the rats, however, and there were no significant differences in zinc content of the animal tissues examined among the three groups of rats fed the dried greens. Rats fed the standard diet with a lower dietary zinc concentration has less zinc per milliliter of blood serum than did rats fed experimental diets containing the plant material. The differences in total carcass zinc between rats fed the standard diet and those fed the ex-

Table V. Mineral Content[a] of Food Consumed, Feces Excreted, and Selected Tissues of Rats Fed a Standard Diet and Diets Containing 50% Dried Turnip Green Tissue[b,c]

	standard diet	turnip green tissue diet		
		NPK	LSS	HSS
		Calcium, mg		
food	2509	3834	5670	7537
feces	1010	2491	3799	5335
% of food[d]	40	65	67	71
carcass	1576a	1313b	1253b	1286b
		Phosphorus, mg		
food	5456	3506	3305	3612
feces	610	1561	1972	2242
% of food	11	45	60	62
carcass	1268a	1052b	1024b	1040b
liver	38.4	43.0	43.0	41.2
kidney	6.3	6.7	5.9	5.6
heart	2.2a	1.7b	1.9b	1.7b
testes	4.7	4.2	4.4	4.0
		Potassium, mg		
food	1828	7260	7766	9287
feces	171	1017	790	925
% of food	9	14	10	10
carcass	957	848	815	859
liver	39.6b	50.4a	49.6a	39.4b
kidney	5.8	6.9	7.1	6.3
heart	2.7	2.3	2.4	2.3
testes	8.3	7.3	8.2	6.9
		Magnesium, mg		
food	151.0	294.0	502.0	635.0
feces	56.0	149.0	241.0	347.0
% of food	37	51	48	55
carcass	61.3a	53.4b	52.3b	51.9b
		Manganese, μg		
food	20670	51160	19060	13330
feces	16040	51990	17710	11860
% of food	78	100	93	89
carcass	211	217	166	215
liver	20b	27a	24a	24a
kidney	2.8	2.4	2.6	2.4
		Iron, μg		
food	26150	68330	99010	138810
feces	12700	59447	85560	125720
% of food	49	87	86	91
carcass	7374	6861	6595	6455
liver	268c	652a	622a	475b
kidney	72	88	87	81
heart	45	37	35	36
testes	21	26	24	24
serum	4.1	5.1	4.2	4.1
		Zinc, μg		
food	8630	18830	97020	111380
feces	4840	15410	87510	91060
% of food	56	82	90	82
carcass	4104a	3766b	3519b	3628b
liver	294	329	329	271
kidney	47	48	52	47
heart	16	13	13	12
testes	53	44	49	43
serum	1.2b	1.5a	1.4a	1.4a
		Copper, μg		
food	2534	1705	1850	2220
feces	1681	1473	1569	1927
% of food	66	86	85	87
carcass	345a	246b	264b	267b
liver	62a	35b	33b	32b
kidney	10.7a	8.9b	9.5ab	9.6ab
heart	5.1a	2.8b	2.5b	2.7b
testes	3.7a	3.2ab	3.4ab	2.9b
serum	1.2a	0.9b	0.8b	0.9b
		Cadmium, μg		
food			782	1096
feces	28	8	469	836
% of food			60	76
carcass	55.2	80.6	85.3	71.0
liver	0.7d	4.3c	9.1b	13.6a
kidney	0.4b	0.4b	2.4a	2.7a
		Chromium, μg		
food	2167	2299	2313	2424
feces	187	713	850	965
% of food	9	31	37	40
carcass	311	154	334	256
liver	20a	15b	15b	18a
kidney	3.9a	1.5b	1.5b	3.9a
heart	2.0a	2.1a	1.5b	1.9a
		Lead, μg		
food	667	4152	2821	3686
feces	405	1855	1930	2650
% of food	61	45	68	72
carcass	375	274	488	386
liver	7.6b	4.3b	15.9a	5.6b
kidney	1.4b	3.5a	3.2a	3.5a
		Nickel, μg		
food	2400	3700	3238	5616
feces	85	1535	2812	2875
% of food	4	41	87	51
carcass	480	348	365	375
liver	2.9	9.8	15.8	12.1

[a] Values are presented as averages per rat of total mineral content of food consumed, feces excreted, and of the tissues analyzed. [b] Turnip greens grown on soils treated with conventional fertilizer (NPK), low sewage sludge (LSS), and high sewage sludge (HSS). [c] In each row, values having no common letter are significantly different at $P \leqslant 0.05$. [d] Fecal excretion of each mineral expressed as percent of intake from food.

perimental diets were related to differences in body weight.

The data in Table V suggest that copper in the dried turnip greens was not as readily available to the rats as that from the copper sulfate added to the standard diet. Total copper content of the carcass, and especially that of the liver, heart, and serum, was less in rats fed the experimental diets containing dried greens than in those fed the standard diet. There were some differences in copper content of the three lots of greens (Table III) which were related to soil treatments. The similarities of tissue mineral content among rats fed the three lots of the plant material, however, indicate an effect of turnip green tissue rather than soil treatment effects.

Cadmium from the sludge-treated soil was taken into the plant material and subsequently accumulated in liver and kidney tissues of rats fed the dried greens. Though much of the dietary cadmium was excreted in the feces, significantly more of the metal was found in tissues of animals fed diets containing turnip greens than in tissues of rats fed the standard diet. Furthermore, liver cadmium content was higher in rats fed the greens grown on the HSS plot than in livers of those fed the greens grown on the LSS plot. Though cadmium concentration in the diet containing greens produced with NPK fertilizer was too low for detection (0.05 mg), livers of rats given this diet also had more cadmium than did the same tissue of rats given the standard diet. The inorganic fertilizer was not analyzed for cadmium but may have contained some cadmium as has been shown by several workers (Lee and Keeney, 1975; Stenstrum and Vahter, 1974). Cadmium may be ubiquitously present in the soil in the area where these turnip greens were produced and the turnip greens may serve as an effective instrument of transfer from soil to animal tissue.

Interpretation of data for chromium, lead, and nickel content of the animal tissues with respect to application of sludge to the soil on which the greens were grown is difficult because of contamination of the diets mentioned above. Excretion of all three of these metals in the feces was much higher in rats fed diets containing 50% of dried greens than in those fed the standard diet in spite of the fact that dietary differences in chromium and nickel content were relatively small. This along with high excretion rates for other elements may indicate a general laxative effect (and consequent reduction in mineral absorption) associated with the high level of dried plant material in the diet.

SUMMARY

Application of sewage sludge to the land resulted in growth of turnip greens at least as vigorous as that obtained with conventional fertilizer (NPK). Total ash content of above-ground plant material was higher in greens grown on sludge than in those grown on NPK and was higher with the high application sludge (HSS) than with the lower level (LSS). Inclusion of the dried material as 50% of the total weight of diets fed to weanling rats had some adverse effects on growth performance. This result could be due to decreased digestibility and/or a laxative effect of the greens. The only differences in rat tissue content of the elements analyzed that appeared to be attributable to sludge treatment were elevated levels of cadmium in liver and kidney.

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Comparative Phytotoxicity of Several Nitrification Inhibitors to Soybean Plants

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Soybeans respond to N fertilization on calcareous arid region soils in Iran but the magnitude of response may vary with N source. Therefore, to select the most suitable nitrification inhibitor, the relative toxicity of nitrapyrin (2-chloro-6-(trichloromethyl)pyridine), sulfathiazole, dicyandiamide, and sodium diethyldithiocarbamate to soybean (*Glycine max* (L.) Merr.) seedlings was studied in a greenhouse experiment. Visual symptoms of nitrapyrin toxicity appeared as leaf curling and tendril type of stem growth. Toxicity of other inhibitors appeared as chlorosis of older leaves, followed by necrotic patches on leaf margins. A restriction in root growth and suppression of fresh and dry weights were the most uniform characteristics of nitrapyrin, sulfathiazole, and dicyandiamide toxicities. However, nitrapyrin was more toxic than the other two inhibitors. Diethyldithiocarbamate did not significantly affect growth at any concentration. Probably, restriction in main root elongation and reduction in lateral root formation, accompanied by reduced water and nutrient absorption were responsible for growth suppression caused by the first three inhibitors. Nitrapyrin further curtailed water and nutrient uptake by inducing tumorous root growth.

Nitrogen fertilizers are subject to many chemical and biological changes in soils, often resulting in significant N losses. Nitrogen losses not only reduce the amount of available N to plants, but also increase the potential for nitrate pollution of surface and ground water (Hill and McCague, 1974; Gentzsch et al., 1974). The other aspect of the intensive N fertilization is possible accumulation of high levels of nitrate in plants. High nitrate levels in fresh vegetables may be hazardous to human health (Maynard et al., 1976) and in forages may be toxic to livestock (Houston et al., 1973). Furthermore, high nitrate contents in forage may represent another potential health hazard when they are ensiled. Denitrification leads to the formation of NO, NO_2, and N_2O_4 which can be lethal to man and animals (Viets, 1965). If the oxidation of applied ammonium can be inhibited, many of these potential problems can be reduced.

In recent years, numerous chemicals have been tested as nitrification inhibitors (Bundy and Bremner, 1973; Gasser, 1970; Goring, 1962a,b; Hauck, 1972; Kapustra and Varsa, 1972; Prasad et al., 1971). However, nitrapyrin (2-chloro-6-(trichloromethyl)pyridine) has been studied in more detail and is the most effective nitrification inhibitor available (Bundy and Bremner, 1973; Patrick et al., 1968).

Mills et al. (1976) and Moore (1973) noted reduced nitrate content in plants under ammonium-N nutrition treated with nitrapyrin. Several workers (Kapustra and Varsa, 1972; Moore, 1973; Patrick et al., 1968; Swezey and Turner, 1962) reported reduced N losses and improved N efficiency with subsequent increased crop production with

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