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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nitrapyrin. In contrast, many researchers have found that nitrapyrin had either no effect (Mills et al., 1976) or produced toxic effects on crops tested (Lynd et al., 1967; McKell and Whalley, 1964; Mills et al., 1973; Parr et al., 1971; Rieck and Lynd, 1967; Sander and Barker, 1978). Geronimo et al. (1973) observed that a safe soil concentration of nitrapyrin for soybean (*Glycine max* (L.) Merr.) was 10 ppmw, which is well above the concentration resulting from application of highest recommended rate. Riley and Barber (1970) reported that yield of soybean shoot was reduced with nitrapyrin concentrations of 8 and 20 ppm and its morphology was changed with concentration as low as 1 ppm. The nature and severity of nitrapyrin toxicity varies with concentration (Geronimo et al., 1973; Mills et al., 1973), plant species (Geronimo et al., 1973; Osborne, 1977; Sander and Barker, 1978), and soil properties (Geronimo et al., 1973). Moreover, the effectiveness of nitrapyrin as inhibitor of nitrification in soil depends greatly upon the type of soil studied (Bundy and Bremner, 1973; Goring, 1962a).

Soybeans respond to N fertilization on calcareous arid region soils in Iran (Behran et al., 1979). However, the magnitude of response may vary with N sources. To select the most suitable nitrification inhibitor, the comparative phytotoxicity of nitrapyrin, sulfathiazole, dicyandiamide, and sodium diethyldithiocarbamate with respect to the growth and development of soybean plant was studied in a greenhouse experiment.

MATERIALS AND METHODS

The surface 20-cm layer of alluvial calcareous silty clay loam with a pH of 7.9 (saturated paste), 1.15% organic matter, 0.07% total N, and 4 ppm $NaHCO_3$-extractable P was used for this study. The soil, classified as Calcixerollic Xerochrept was air-dried and crushed to pass a 2-mm screen. Nitrapyrin was added as emulsion diluted with water. Sulfathiazole, dicyandiamide, and sodium diethyldithiocarbamate were used as aqueous solutions. Dilutions were made so that the desired concentrations could be applied in 10-mL aliquots. Inhibitors were added to the surface of 1000-g, air-dried soil samples to provide the following concentrations: 0, 5, 10, 20, 40, and 100 ppm nitrapyrin and 0, 10, 20, 40, 100, and 200 ppm from the other inhibitors. Soil amended with various nitrification inhibitors was allowed to dry for several hours and then was thoroughly mixed.

Eight soybean seeds, cv. Clark, were planted per pot and seedlings were thinned to two plants per pot 7 days after emergence. Pots were irrigated with distilled water to near field capacity by weight as needed and grown for 54 days. The experiment was a completely randomized design with three replications. At harvest, seedlings were cut at the soil surface and top fresh weight was determined. Roots were washed thoroughly with distilled water, using a wire mesh screen to hold them in place. Tops and roots were dried to a constant weight at 70 °C; dry weights were recorded and tops were ground in a Wiley mill to pass a 40-mesh, stainless-steel screen. Total N was determined by microKjeldahl method.

The data were subjected to the analysis of variance and Duncan's multiple range test was used to compare significant differences between means.

RESULTS AND DISCUSSION

At harvest, soybean seedlings were visually evaluated for severity of toxicity symptoms. The plants developed toxicity symptoms similar to those of black locust (*Robinia pseudoacacia*) treated with nitrapyrin as described by Lynd et al. (1967). The typical symptoms consisted of leaf curling and cupping, internode elongation, and profuse

Table I. Effect of Various Nitrification Inhibitors on Top Fresh Weight and Top and Root Dry Yields of Soybean Plants[a]

	sulfathiazole			dicyandiamide			sodium diethyldithiocarbamate			2-chloro-6-(trichloromethyl)pyridine		
		dry wt, g/pot			dry wt, g/pot			dry wt, g/pot			dry wt, g/pot	
inhibitor level, ppm	fresh wt, g/pot	tops	roots	fresh wt, g/pot	tops	roots	fresh wt, g/pot	tops	roots	fresh wt, g/pot	tops	roots
0	9.6a	2.74a	1.21a	8.4a	2.23a	1.01a	7.0a	1.79a	0.93a	9.7a	2.76a	1.32a
5										7.5b	1.57b	0.85b
10	8.4a	2.25ab	0.95ab	7.3ab	2.05ab	1.01a	7.0a	1.71a	0.88a	5.1c	1.06bc	0.61b
20	6.5b	1.72bc	0.71bc	7.3ab	2.02ab	0.91a	6.6a	1.66a	1.70a	3.9c	0.89c	0.73b
40	4.8b	1.22c	0.47c	6.3b	1.61b	0.73ab	6.1a	1.52a	0.85a	1.6d	0.32d	0.25c
100	1.0c	0.31d	0.17d	4.4c	0.98c	0.50bc	6.7a	1.42a	0.89a	0.8d	0.14d	0.12c
200	0.4c	0.15d	0.03d	2.7d	0.56c	0.28c	6.3a	1.45a	0.85a			

[a] Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table II. Soil Concentration of Various Nitrification Inhibitors Required to Reduce Fresh Weight and Dry Yields by 50%

	ED_{50},[a] ppm		
		dry wt	
nitrification inhibitor	fresh wt	tops	roots
sulfathiazole	42	33.5	29.5
dicyandiamide	108	86.0	97.0
sodium diethyldithiocarbamate	>200	>200.0	>200.0
2-chloro-6-(trichloromethyl)pyridine	12	8.0	14.0

[a] Soil concentrations required to reduce fresh weight and top and root dry yields by 50%.

deformed leaf clusters at the shoot apex, presumably due to the abnormal meristematic tissue development. The symptoms were most acute with the highest rate of nitrapyrin. Mills et al. (1973) noted that garden pea (*Pisum sativum* L.) seedlings developed curling of the leaf margins with nitrapyrin application of 10 ppm and very pronounced curling above 10 ppm, while bean (*Phaseolus vulgaris* L.) showed a temporary manifestation of nitrapyrin toxicity on the primary leaves. In a subsequent study, Mills et al. (1976) reported that radish (*Raphanus sativus* L.) did not appear to be injured at concentrations as high as 50 ppm nitrapyrin in soil.

With regards to the other three inhibitors, toxicity symptoms first appeared as chlorosis of the older leaves, followed by necrotic patches on lamina or leaf marginal tissues. In general, phytotoxicity increased with increasing inhibitor levels. Reddy (1964) observed that dicyandiamide was toxic to corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), tomato (*Lycopersicon esculentum* Mill.), and oats (*Avena sativa* L.). Toxicity symptoms appeared as white burns on the leaf tips or margins with severe withering of leaves from the tips downward with 25 ppm of inhibitor.

Soybean roots showed no toxicity symptoms from diethyldithiocarbamate at the levels applied. Dicyandiamide at 100 ppm rate slightly reduced main root elongation and suppressed lateral root formation at 200 ppm. Sulfathiazole and nitrapyrin drastically reduced both main root elongation and lateral root development; the suppression was most pronounced at the highest chemical concentrations. In addition, nitrapyrin-treated soybean plants had stubby and club-like root systems. A decrease in main root elongation and lateral root formation due to nitrapyrin has been reported by Lynd et al. (1967). Riley and Barber (1970) found that 8 and 20 ppm nitrapyrin caused soybean roots to be stubby and form club-like swelling, particularly just behind the root tips.

Fresh weight and top and root dry yields, as affected by various inhibitor, are shown in Table I. Lines of best fit were plotted from the data in Table I and ED_{50} values for each chemical were calculated (Table II).

Fresh weight and dry matter production were not significantly affected by any level of diethyldithiocarbamate, but were generally suppressed with increasing rates of the other inhibitors (Table I). Soybean plants showed a slight but nonsignificant drop in growth with 10 ppm sulfathiazole. However, growth was significantly reduced with the higher concentrations. For sulfathiazole, the most substantial decrease in soybean yields occurred on soil treated with 100 and 200 ppm. For example, 32, 37, and 41% reductions in fresh weight and top and root dry yields were obtained with 20 ppm sulfathiazole, whereas, with 100 ppm, those reductions were 90, 89, and 86%, respectively.

Dicyandiamide applied at 10 or 20 ppm did not have any significant effect on soybean growth as compared to that of the untreated check (Tabled I). However, higher rates significantly suppressed fresh weight and top dry yields. Furthermore, there was a gradual but insignificant decrease in soybean root growth with increasing dicyandiamide from 0 to 40 ppm with more abrupt changes at the higher concentrations. Hurkowska et al. (1976) reported that 25 ppm of dicyandiamide was not toxic to oats. In contrast, Reddy (1964) noted that 25 ppm of dicyandiamide severely depressed the growth of corn, cotton, tomato, and oat plants.

A severe reduction in fresh or dry matter production was one of the most uniform characteristics of nitrapyrin toxicity. Application of 5 ppm to soil significantly decreased soybean growth relative to that of the control (Table I). However, the suppressing effect of nitrapyrin was more pronounced with the higher application rates (40 and 100 ppm) than with the lower ones. A reduction in plant growth from nitrapyrin addition to soil has been also reported by Geronimo et al. (1973) and Riley and Barber (1970) in soybean, by Mills et al. (1973) in peas, and by Sander and Barker (1978) in cucumber (*Cucumis sativus* L.). On the other hand, with corn some workers (Dibb and Welch, 1976; Mills et al., 1973) observed a general trend of increased plant growth with nitrapyrin application up to 50 ppm. Dibb and Welch (1976) stated that corn yield response at 10 and 20 ppm nitrapyrin has resulted from the growth stimulant caused by the chemical itself, increased efficiency of nitrification inhibition or NH_4^+-N availability through normal ammonification. In a later experiment, they reported a consistent decrease in corn yield with an increasing N:K ratio, where NH_4 was the major source of N absorbed.

In the present study, nitrapyrin reduced soybean top growth more than root growth, whereas root growth was suppressed slightly more than top growth by sulfathiazole. For instance, the root/top ratios associated with 0, 10, 20, and 40 ppm of nitrapyrin were 0.48, 0.58, 0.82, and 0.78,

Table III. Effect of Various Nitrification Inhibitors on the Nitrogen Concentration and Nitrogen Uptake by Soybean Plants[a]

	sulfathiazole		dicyandiamide		sodium diethyldithiocarbamate		2-chloro-6-(trichloromethyl)pyridine	
	nitrogen in plant top		nitrogen in plant top		nitrogen in plant top		nitrogen in plant top	
inhibitor level, ppm	concn, %	uptake, mg/pot	concn, %	uptake, mg/pot	concn, %	uptake, mg/pot	concn, %	uptake, mg/pot
0	1.83d	50.10a	1.79c	39.73a	2.54a	45.40a	1.83e	50.53a
5							2.94d	46.13ab
10	1.99d	44.40ab	1.99c	40.60a	2.26a	38.53a	3.52c	37.63bc
20	2.49c	42.60ab	2.17bc	43.80a	2.48a	41.13a	3.40c	30.43c
40	2.99b	36.07b	2.51b	40.73a	2.59a	39.53a	4.31b	13.83d
100	4.52a	13.97c	3.56a	34.80a	2.37a	33.73a	5.47a	7.43d
200	5.87a	8.80d	3.75a	20.87b	2.46a	35.40a		

[a] Means within a column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

respectively, while those ratios for the same levels of sulfathiazole were 0.44, 0.42, 0.41, and 0.38, respectively.

The data in Table II indicate that nitrapyrin was the most phytotoxic inhibitor to soybeans. Sulfathiazole showed greater toxicity than that of dicyandiamide and soybean plants exhibited greatest tolerance to sodium diethyldithiocarbamate. The sensitivity of soybeans to nitrapyrin was about 2–3.5 times greater than that to sulfathiazole and ED_{50} values for dicyandiamide were about 2.5–3 times greater than those for sulfathiazole.

Percent N and N uptake by soybean tops varied considerably, depending on the nitrification inhibitor used (Table III). All inhibitors, with the exception of diethyldithiocarbamate, increased percent N and decreased N uptake. However, soybean seedlings on soil treated with diethyldithiocarbamate were not significantly different in either percent N or N uptake.

Percent N in the plant tops was little affected by 10 ppm of sulfathiazole or 20 ppm of dicyandiamide but was significantly increased with increasing concentrations up to 100 ppm (Table III). No marked response was noted from additional application of the chemicals. The percent N significantly increased with each increment nitrapyrin.

In conclusion, it appears that growth suppression caused by sulfathiazole, dicyandiamide, and nitrapyrin application may possibly have resulted from reduced lateral root formation and main root elongation accompanied by reduced water and nutrient absorption. Moreover, formation of tumorous root growth of nitrapyrin-treated soybeans further interfered with water and nutrient uptake. The fact that 6-chloropicolinic acid is the initial and principal metabolite present after the application of nitrapyrin suggests that some of the toxicity observed with nitrapyrin is probably due to 6-chloropicolinic acid (Geronimo et al., 1973). This compound is absorbed by plants and is generally distributed throughout the plant (Meikle and Redeman, 1966).

In Iran, soybeans, as well as some other leguminous crops, respond to N fertilizers applied to calcareous arid region soils; the magnitude of response might be greatly affected by N source. Furthermore, based on previous work in our laboratory we have good reason to believe that the lowest nitrapyrin concentration to inhibit nitrification in our fine-textured calcareous soils for reasonable lengths of time is 10 ppm which is well above the highest recommended rate. Therefore, it is unlikely that it could be used with soybeans or other sensitive crops. It should be noted that nitrification inhibitors, especially nitrapyrin, may be used in conjunction with NH_4 fertilizers in legume-grass pastures, therefore, their possible greater toxic effects on legume in such mixtures should be considered. In the present study, although sodium diethyldithiocarbamate had no suppressing effect on soybean growth, it cannot be recommended as the most suitable nitrification inhibitor, since it is one of the poorest nitrification inhibitors in most soils (Bundy and Bremner, 1973).

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