Fate of Ionic Thiocyanate (SCN^-) in Soil

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Ionic thiocyanate (SCN^-) additions to soil result from anthropogenic and natural sources; however, little is known concerning the fate of this anion in soil. Six soils were amended with SCN^- at concentrations expected from *Brassica* tissues. Thiocyanate in 0.005 M $CaCl_2$ extracts was quantified using ion chromatography. The decrease in SCN^- concentrations with time for incubations conducted at or below 30 °C was positively correlated with soil organic carbon content in five of six soils. Autoclaving soils prior to incubation and adding NaN_3 or allyl isothiocyanate slowed SCN^- disappearance. Waterlogged conditions slowed disappearance initially, but the trend was not observed at 72 h. Extract concentrations of SCN^- also decreased when the soils were incubated at higher temperatures of 50 or 60 °C. Microbial degradation of SCN^- is the main factor responsible for SCN^- disappearance at or below 30 °C. At higher temperatures decreases in extractable SCN^- involve sorption or degradation by abiotic catalysis.

INTRODUCTION

Anthropogenic additions of ionic thiocyanate (SCN^-) to soil occur through direct application in herbicidal formulations (such as amitrol-T; a mixture of amino-1,2,4-triazole and NH_4SCN) or result as byproducts from industrial processes. Natural sources of SCN^- in the environment include damaged or decaying tissues of plants from the Brassicaceae. Indole and *p*-hydroxybenzyl glucosinolates contained in *Brassica* spp. tissues are hydrolyzed by the enzyme thioglucoside glucohydrolase (EC 3.2.3.1) to produce unstable isothiocyanate intermediates which spontaneously form SCN^- (Figure 1) (van Etten and Tookey, 1979). Concentrations of SCN^- were recently quantified in soils amended with defatted seed meal of *Brassica napus* L. (Brown et al., 1991).

Because different glucosinolates produce a variety of allelochemicals during their breakdown, in addition to SCN^-, interest has been generated in using *Brassica* residues as a substitute for synthetic pesticides. Although SCN^- is less toxic than related cyanides and isothiocyanates, biological activity has been demonstrated (Ju et al., 1983; Beekhuis, 1975; Wood, 1975; Wu and Basler, 1969; Smith et al., 1945). To effectively and safely implement such natural pest control strategies, it is essential to understand the fate of allelochemicals such as SCN^- in soil.

As a result of environmental concerns regarding pesticide use and SCN^- in industrial effluents, studies examining the impact of SCN^- on soil microorganisms have been conducted (van Schreven et al., 1970; Smith et al., 1945) and specific organisms degrading SCN^- have been identified (Betts et al., 1979; Stafford and Callely, 1969; Happold et al., 1954). However, SCN^- behavior in soil is largely uncharacterized. Our objective was to quantify SCN^- residence times in different soils and determine if microbiological degradation controlled the observed disappearance.

MATERIALS AND METHODS

Soils. Soils with a range in organic C, pH, and particle size distribution were selected (Table I). Surface soil samples (0–15 cm) were collected, air-dried, and sieved (2 mm). Soil characteristics were determined according to the following methods:

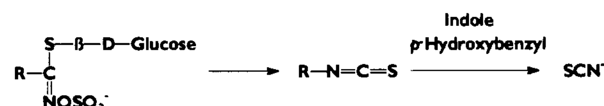

Figure 1. Pathway for the production of SCN^- from specific glucosinolates contained in *Brassica* spp. tissues.

pH by glass electrode (1:1 soil to water), organic C by a modified Walkley-Black method (Nelson and Sommers, 1982), and total C and N by Dumas combustion (LECO CHN-600 determinator, St. Joseph, MI). Particle size distributions for Portneuf, Palouse, Latahco, Feltham, and Fenn soils were determined by the hydrometer method and for the unnamed silt loam soil by the pipet method (Gee and Bauder, 1986). Moisture content at a water potential of –0.033 MPa was determined using a ceramic pressure plate (Klute, 1986).

SCN^- Analysis. Thiocyanate in soil extracts was quantified using ion chromatography (IC) (Brown and Morra, 1991). Instrumentation consisted of a Dionex 4000i ion chromatograph equipped with an AS5 column, anion micromembrane suppressor, and conductivity detector in combination with a 4270 Spectra Physics integrator. Eluent was pumped at a flow rate of 2 mL/min and contained 4.3 mM $NaHCO_3$, 3.4 mM Na_2CO_3, and 0.7 mM 4-cyanophenol. Thiocyanate was extracted from 8 g of soil by shaking for 30 min with 20 mL of 0.005 M $CaCl_2$. A minimum of three replicates were extracted for all analyses. The resulting soil extract was filtered through Whatman No. 42 filter paper, refiltered through a 0.45-μm membrane syringe filter (Gelman, Supor-450), and refrigerated until IC analysis. Calibration curves for SCN^- were constructed for each group of soil extracts using aqueous solutions of KSCN (Baker, Phillipsburg, NJ).

Incubation Procedures. Air-dried soil samples (8 g) were placed in 50-mL centrifuge tubes and amended with KSCN solutions at a rate of 5.8 μg of SCN^-/g of soil, an amount corresponding to that observed previously in rapeseed meal-amended soil (Brown et al., 1991). A moisture content equivalent to that present at –0.033 MPa was maintained in all soils except for those used in the waterlogged experiments. All soil samples were incubated in the dark at 30 °C, except as noted. Soil lacking KSCN but incubated under the same moisture and temperature conditions served as a control. Thiocyanate disappearance was monitored in four soils at 10 intervals over a 120-h time period. Aqueous solutions lacking soil were prepared as above and incubated along with soil treatments.

The effects of temperature were determined using six soils. Thiocyanate was quantified at 72 h for temperatures of 5, 12, 20, 30, 40, 50, and 60 °C. To check for thermal degradation of SCN^-, controls without soil were incubated at 30 and 60 °C. All samples incubated above 30 °C were covered with 60-gauge D955 shrink wrap film (W. R. Grace, Cryovac Division) to prevent drying. To

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Table I. Characteristics of Soils Used in SCN⁻ Incubations

soil		pH	organic carbon, g/kg	total carbon, g/kg	total nitrogen, g/kg	clay, g/kg	sand, g/kg
series	subgroup						
Feltham ls	Xeric Torriorthents	6.6	6.3	8.2	0.8	40	830
Fenn sicl	Chromic Pelloxerert	5.6	27.0	27.0	2.1	343	193
Palouse sil	Pachic Ultic Haploxeroll	5.7	19.2	19.5	1.6	211	88
Latahco sil	Argiaquic Xeric Argialboll	6.1	41.0	41.0	3.9	159	122
Portneuf sil	Durixerollic Calciorthid	7.3	11.6	15.6	1.7	157	48
unnamed sil	Typic Vitricryand	6.4	15.0	15.0	1.8	117	190

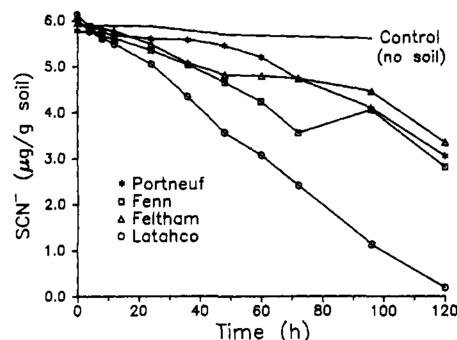

Figure 2. Decrease in soil SCN⁻ concentrations with time of *incubation.*

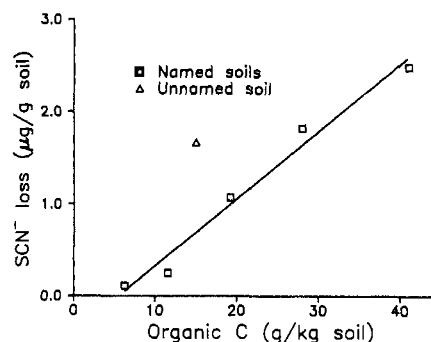

Figure 3. Net decrease in extractable SCN⁻ concentrations as a function of soil organic C content when soils were amended with 5.8 μg of SCN⁻/g of soil and incubated at 20 °C for 72 h. Linear regression ($Y = 0.073X + 0.40$, $r^2 = 0.98$, $P > F = 0.002$) was calculated using mean SCN⁻ concentrations for named soils only.

determine if extraction of SCN⁻ was enhanced by addition of an anion that would strongly compete for anion adsorption sites, samples were incubated at 30, 50, and 60 °C and extracted with a solution containing 0.01 M KH_2PO_4 and 0.005 M $CaCl_2$. Four soils were used, and incubation parameters were identical to those of other temperature studies.

Treatments to inhibit biological activity and any associated degradation of SCN⁻ were applied to six soils. Treatments consisted of steam sterilization by autoclaving and additions of sodium azide (12 μmol/g of soil), allyl isothiocyanate (300 nmol/g of soil added as a sonified aqueous solution), toluene (0.5 mL), and chloroform (0.1 mL). Allyl isothiocyanate was chosen because isothiocyanates are also produced in soils amended with rapeseed meal (Brown et al., 1991). Controls consisted of treated soils minus SCN⁻. Treatments containing biological inhibitors were incubated for 24 and 72 h, and all were capped or covered. In waterlogged experiments, 5.5 mL of total water was added to each sample except the unnamed soil, to which 7.0 mL was added due to the high moisture-holding capacity of the volcanic ash contained in that soil. Incubation times were 24 and 72 h.

RESULTS AND DISCUSSION

Gleen (1949) showed that SCN⁻ disappearance in soils is enhanced by additions of carbohydrates, non-sulfur-containing amino acids, and the presence of alkaline conditions. Increased SCN⁻ disappearance after perfusion led the author to suggest that the disappearance was biological in nature. Since then, a variety of bacteria have been reported to degrade SCN⁻. Thiocyanate-degrading *Pseudomonas* spp. have been isolated from sewage waters of coke factories (Putilina, 1961) and activated sludge tanks (Stafford and Callely, 1969). *Thiobacillus* spp. that degrade SCN⁻ have been isolated from such varied sources as marine mud, cattle manure, and pond water (Smith and Kelly, 1988), as well as activated sludge (Katayama and Kuraishi, 1978), sewage effluents, and contaminated well water (Happold et al., 1954). Betts et al. (1979) isolated a SCN⁻-degrading *Arthrobacter* sp. from soil adjacent to a railroad embankment.

Our data are consistent with the idea that SCN⁻ disappearance in soil is microbially mediated. Soil extracts showed a decrease in SCN⁻ concentration as a function of incubation time (Figure 2). Solutions containing no soil showed almost no decrease in SCN⁻ concentration in 96 h (Figure 2). The most rapid disappearance occurred in the Latahco soil, where essentially no extractable SCN⁻ remained after 120 h.

Disappearance was highly correlated with soil organic C content in five of six soils (Figure 3). The only exception was the unnamed volcanic ash influenced soil. Clay content did not show a significant correlation with SCN⁻ loss at 20 °C for the five soils (*F* test, $P = 0.34$). The influence of organic carbon is illustrated by the Latahco and Palouse silt loams, which are adjacent soil series with similar mineralogical characteristics. The Latahco soil, with more than twice the organic carbon content and less clay than the Palouse, had over twice the disappearance of SCN⁻ in 72 h (Figure 3).

This correlation was only observed at or below 30 °C, which suggests that SCN⁻ losses were related to the amount of C available to support microbial activity rather than SCN⁻ sorption to the organic C fraction of the soil. The three soils lowest in organic C (Feltham, Portneuf, and unnamed) have C:N ratios of less than 9. The remaining three soils (Latahco, Palouse, and Fenn) have C:N ratios exceeding 10. This relationship indicates that C is limiting microbial activity for those soils having the lowest rates of SCN⁻ disappearance and that inorganic nutrient limitations dominate in those soils higher in organic C content.

Thiocyanate disappearance was slowed in soils incubated for 24 h in the presence of NaN_3 (Table II). With the exception of the Feltham soil, this trend was also evident at 72 h. Soils autoclaved prior to incubation with SCN⁻ showed either no change or a reduction in the rate of SCN⁻ disappearance in 24-h incubations. Sodium azide treatment of soil at a rate 3.08 mmol/kg of soil reduces bacterial and fungal populations in some soils but does not completely sterilize the soil (Wolf et al., 1989). Autoclaving soils for one 2-h time period has been shown to reduce bacterial numbers by 10^5 and fungal numbers by 10^3 but not to eliminate the populations (Wolf et al., 1989). The reduction in microbial activity as produced through NaN_3 addition or autoclaving resulted in less SCN⁻ degradation

Table II. Extractable SCN^- Determined after SCN^--Amended Soils Were Incubated under Specified Conditions

	SCN⁻ extracted,[a] μg/g of soil					
	Portneuf	Fenn	Feltham	Latahco	Palouse	unnamed
			24 h			
no inhibitor	5.62	5.48	5.48	5.07	5.22	4.79
toluene	5.50	4.99[b]	5.36	4.40[b]	5.10	4.64
chloroform	5.25[c]	4.32[c]	4.69[c]	3.39[c]	4.60[c]	4.58[b]
autoclaved	5.60	5.66	5.73[c]	5.51	5.59[b]	5.12[c]
NaN_3	5.87[b]	5.96[b]	5.79[c]	6.03[c]	6.09[c]	5.12[c]
allyl ITC[d]	6.12[c]	5.42	5.84[c]	5.77[b]	5.81[c]	5.31[c]
waterlogged	5.95[c]	5.83	5.88[c]	5.62	6.05[c]	5.87[c]
			72 h			
no inhibitor	4.72	3.56	4.73	2.41	4.62	4.53
NaN_3	4.78	4.62[c]	4.41[c]	4.84[c]	4.91[b]	4.86[c]
allyl ITC[d]	5.56[c]	4.87[c]		3.47[c]	6.08[c]	5.26[c]
waterlogged	4.49[b]	3.55	3.33[c]	4.87[c]	4.41	4.36

[a] LS means calculated with SAS using the GLM procedure. [b,c] Significant difference at the 0.10 and 0.05 probability levels, respectively, compared to no inhibitor at the specified incubation time. [d] Allyl ITC, allyl isothiocyanate.

and greater extractable concentrations, again implicating the role of microbial activity in SCN^- disappearance in soil.

The addition of allyl isothiocyanate effectively inhibited SCN^- disappearance in four of the soils in 24-h assays and in all five soils assayed after 72 h of incubation (Table II). This has relevance to situations in which *Brassica* spp. tissues, which contain a variety of glucosinolates, are amended to soil. Glucosinolates contained within those tissues will produce isothiocyanates (Figure 1) which act as nonspecific biocides by reacting with amine and sulfhydryl groups in proteins (Wood, 1975). Glucosinolates having indole and *p*-hydroxybenzyl groups attached to the thioglucose moiety produce unstable isothiocyanate intermediates which spontaneously degrade to SCN^-. However, isothiocyanates formed from other glucosinolate precursors have longer residence times. Isothiocyanates negatively impact components of the microbial population and, as shown here, inhibit SCN^- degradation rates. The growth of several bacterial cultures was shown to be inhibited by allyl isothiocyanate and allyl isothiocyanate-containing brown mustard extracts (Kanemaru and Miyamoto, 1990). Potential hydrolysis products of isothiocyanates such as COS, CS_2, and H_2S (Bailey et al., 1961; Challenger, 1959) also have allelochemic effects. We have previously shown the simultaneous production of isothiocyanate and SCN^- in soil amended with defatted seed meal of *B. napus* L. (Brown et al., 1991).

Incubation of the soils under waterlogged conditions slowed the disappearance of SCN^- in 24-h assays of Portneuf, Feltham, Palouse, and the unnamed silt loam, but this trend was not observed after 72 h (Table II). *Pseudomonas* spp. and *Thiobacillus* spp. have been shown to metabolize SCN^- under both aerobic and anaerobic conditions (Stafford and Callely, 1969; Youatt, 1954), whereas an *Arthrobacter* sp. that metabolized SCN^- could only be grown aerobically (Betts et al., 1979). Although the pathway for the microbial degradation of SCN^- is not known, saturated paddy soils amended with SCN^- were found to evolve COS (Minami and Fukushi, 1981) exclusive of several other sulfur gases (Minami, 1982).

In contrast, toluene addition to the assay mixtures showed a slight tendency to increase SCN^- disappearance ($P = 0.1$) for Fenn and Latahco soils. Chloroform addition produced a much more dramatic increase in SCN^- disappearance for all but the unnamed silt loam (Table II). Although substitutions involving alkyl halides and SCN^- occur, chemical reactions between chloroform or toluene and SCN^- which could account for such losses under the relatively mild conditions as provided in our assays are unlikely (Ashworth, 1972, 1975; Hughes, 1975).

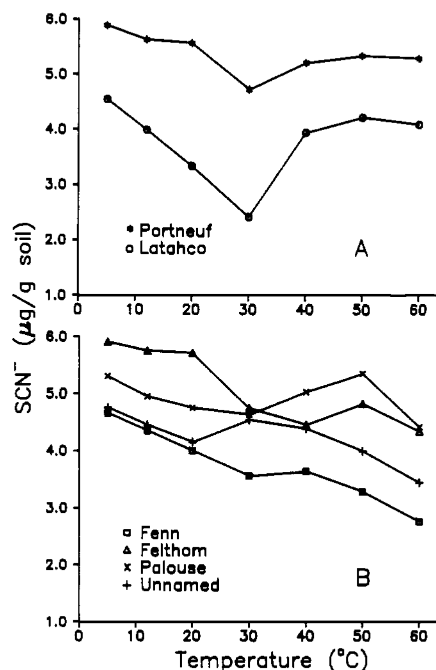

Figure 4. Extractable SCN^- concentrations from soils after incubation at different temperatures for 72 h.

Toluene is not an effective soil sterilent but serves in assays of several hours to inhibit microbial activity (Tabatabai, 1982). Chloroform treatment may reduce microbial numbers in soils but has much less of an impact on bacteria than NaN_3 (Wolf et al., 1989). Toluene and chloroform are ineffective soil sterilents which fail to inhibit microbial activity during the relatively long incubation times of the assays. Increased SCN^- degradation results from the flush of microbial activity which follows partial destruction of the microbial component and the associated release of intracellular nutrients, much like that which occurs in chloroform fumigation techniques (Jenkinson and Powlson, 1976).

The role of microbial activity in SCN^- degradation was also indicated by temperature studies. Soils showed the least disappearance of SCN^- when incubated at 5 °C (except the Palouse, which had approximately the same disappearance at 5 and 50 °C) (Figure 4). Portneuf and Latahco soils showed the largest reductions in extractable SCN^- when incubated at 30 °C (Figure 4A). *Arthrobacter* sp. and *Thiobacillus* sp. that degrade SCN^- have been reported to have optimum growth at 30 °C (Betts et al., 1979; Happold et al., 1954). However, Stafford and Callely (1969) reported that growth of a SCN^--degrading *Pseudomonas* sp. was maintained at 41 °C. The remaining soils displayed a different pattern in SCN^- disappearance with minima occurring at physiologically permissive temperatures (20–40 °C) as well as at 60 °C (Figure 4B).

The combined data demonstrate that microbial degradation is a major factor in SCN^- disappearance in soil but do not explain all of the observed losses. Decreases in extractable SCN^- at 50 and 60 °C (Figure 4) implicate nonbiological mechanisms for SCN^- disappearance, particularly at higher temperatures. In fact, four of six soils showed maximum SCN^- losses when incubated at 60 °C (Figure 4B), a temperature at which biological degradation would be unexpected. Corresponding reductions of SCN^- concentrations were not found in controls lacking soil,

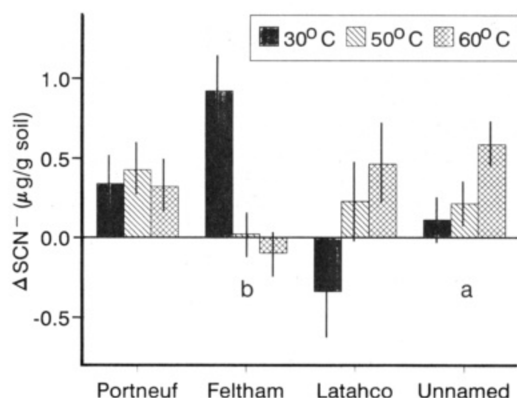

Figure 5. Difference in SCN^- contained in 0.005 M $CaCl_2$ extracts and 0.005 M $CaCl_2$ + 0.01 M K_2HPO_4 extracts obtained from SCN^--amended soils. Quantity bars above the zero line represent additional SCN^- extracted with combined extractant; bars below the zero line represent decreased SCN^- extraction efficiency with the combined extractant. Error bars are ±1.96 standard errors ($P = 0.05$). a and b indicate significant temperature effect at $P = 0.10$ and 0.05, respectively.

indicating that the phenomenon was not caused by the thermal degradation of SCN^-. The reduction in extractable SCN^- must therefore result from decreased extraction efficiency of sorbed SCN^- or its abiotic degradation.

Disappearance by sorption was suggested by the behavior of the unnamed silt loam, which was unusual in that it exhibited a higher reduction in extractable SCN^- than other soils in relation to its organic C content (Figure 3). This soil has a large amount of volcanic ash and a correspondingly high anion-exchange capacity (Wada, 1989). If higher temperatures were causing increased sorption, or increased sorption kinetics, then increased extraction of SCN^- using PO_4 would be expected particularly for the unnamed soil. A clear interpretation of the extraction results was not possible (Figure 5). Two of the four soils, Portneuf and the unnamed silt loam, showed significant differences (F test, $P = 0.05$) when extracted with PO_4 after a 72-h incubation, resulting in higher extraction efficiencies averaged across the three temperatures. There was a trend for increased SCN^- extraction from the unnamed and Latahco soils with increased temperature, but an opposite relationship was observed for Feltham samples and no trend for the Portneuf soil (Figure 5). Even for amounts considered significant, the increased amount of SCN^- extracted is not large enough to explain the decreases in extractable SCN^- observed at higher temperatures (Figure 4). However, it is possible that SCN^- in some soils is not displaced by PO_4, sorption sites were not saturated, or the kinetics of replacement were too slow. Decreased extraction efficiency with increased incubation temperature has been demonstrated with other anions, including PO_4^{3-}, F^-, SO_4^{2-}, and MoO_4^{2-} (Barrow and Shaw, 1977a,b, 1975a,b; Barrow, 1974). Adsorption of SCN^- may become significant at elevated temperatures or in those soils with high concentrations of hydrous oxides of aluminum or iron.

The abiotic degradation of SCN^- catalyzed by soil constituents such as clays and humic materials is also possible and would become more pronounced at higher temperatures. Though the mechanism is not applicable under our assay conditions, photooxidation of SCN^- has been observed (Knoevenagel and Himmelreich, 1976), demonstrating one form of abiotic SCN^- degradation.

Microbial degradation is the major factor affecting SCN^- disappearance in soil incubated at or below 30 °C. Decreases in extractable SCN^- at temperatures above 40 °C result most likely from sorption, but abiotic catalysis of SCN^- degradation is also possible.

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