Effects of the Insecticide Endosulfan on Nitrification in Low pH Agricultural Soils

GLENN W. STRATTON

Environmental Microbiology Laboratory,
Department of Biology,
Nova Scotia Agricultural College,
Truro, Nova Scotia, Canada B2N 5E3

ABSTRACT

Two methods of measuring nitrification in soil bioassays were compared for their sensitivity in estimating toxic effects of endosulfan, a cyclodiene insecticide. Both technical grade endosulfan (95% pure) and endosulfan formulated as a commercial preparation (Thiodan 4EC) were tested in a sandy loam (pH 5.7), a silt loam (pH 5.0), and a clay loam (pH 4.9) soil. Nitrification was assayed using both soil perfusion and batch incubation techniques. There was a significant difference in the nitrification pattern evidenced by the three soils. In the sandy loam soil up to 90% of the added ammonium ion was converted into nitrate within 30 days, while the silt loam required 40 days to achieve similar nitrate levels. Activity in the clay loam was very slow and only 5% of the added ammonium ion was converted into nitrate within 70 days. The nitrification pattern for any given soil was similar when measured by both test methods. At insecticide levels of 10, 50, and 100 ppm of active ingredient, there was no significant difference in toxicity between the commercial preparation and technical endosulfan in 50% of the systems tested. In the remainder, the commercial formulation was usually more toxic. For concentrations of 500 and 1000 ppm of active ingredient, endosulfan supplied as the commercial preparation was consistently more toxic than the technical material when tested toward nitrification. There was also a significant difference between the two incubation methods in their sensitivity to endosulfan. The exact level of toxicity was dependent upon both the soil type and the incubation method used. Generally, endosulfan was more toxic with the batch incubation system and in the silt loam soil. The importance of these results in nitrification bioassays are discussed.

INTRODUCTION

The effects of pesticides on nontarget microbial populations and processes in soil and aquatic ecosystems are an important aspect of research into the environmental impact of these xenobiotics. In particular, microbial processes associated with the nitrogen cycle are often

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chosen as test systems, due to the ecological significance of nitrogen transformations in the biosphere. Of all the biochemical processes in soil, nitrification, particularly autotrophic nitrification, is one of the most sensitive to pesticide stress (Ray and Sethunathan, 1988). This is due to the high degree of sensitivity of the nitrifying bacteria to external influences (Dhanaraj, 1988). This makes nitrification a useful toxicity criterion in environmental impact studies.

In spite of the importance of nitrogen cycling in soils, comparatively little attention has been given to the effects of pesticides on these processes (Lal, 1988). For example, toxicity data specifically for nitrification are still lacking for a number of routinely used pesticides (see reviews by Reddy *et al.*, 1984; Dhanaraj, 1988; Ray and Sethunathan, 1988). Even less attention has been given to the study of pesticide effects toward nitrogen cycling in low pH soils, even though acidic soils are predominant in many areas, including Atlantic Canada. These problems are due, in part, to the lack of standardized methodology in this field (Lal, 1988). There is a general lack of research into factors affecting the sensitivity of bioassay procedures and a lack of comparative studies on bioassay techniques. Methods must be standardized in order to facilitate the comparison of toxicity data obtained from different laboratories.

The purpose of the present study was to compare the sensitivity of two methods used to monitor nitrification in soil bioassays. The insecticide endosulfan, both as the formulated product and as the pure unformulated pesticide, was used as the test compound. Endosulfan is a commonly used insecticide, but few data are available on its effects toward nitrification in soil (Reddy *et al.*, 1984; Dhanaraj, 1988). Few data are also available on the comparative toxicity of formulated and unformulated pesticides (Reddy *et al.*, 1984). Low pH soils were used because they are prevalent in Atlantic Canada. The data presented here will provide important information on bioassay procedures used for measuring nitrification, as well as data on the toxicity of endosulfan toward this process in some low-pH agricultural soils.

MATERIALS AND METHODS

Test Soils

Three test soils were used in the present study—a sandy loam, a silt loam, and a clay loam. Soil samples were collected from the uncut edge of hay fields found near the town of Truro, Nova Scotia, Canada. The characteristics of these soils are summarized in Table I. All experiments employed freshly collected soil. Samples were not allowed to air dry

TABLE I
Characteristics of the test soils

Soil name	Soil separates (%)			pH	Organic matter (%)	NO_3^-–N concentration[a]
	Sand	Silt	Clay			
1. Truro sandy loam—TSL	69	23	8	5.7	3.2	7.2 ± 1.0
2. Stewiacke silt loam—SSL	29	57	14	5.0	5.4	0.5 ± 0.4
3. Queen's clay loam—QCL	40	30	30	4.9	3.5	2.8 ± 1.1

[a] Background concentration of NO_3^-–N; microgram per gram oven dry weight soil; mean ± the standard deviation.

and were kept in airtight containers until their moisture content was determined. The amount of soil used in each experiment was then standardized to be equivalent to the oven dry weight required for each particular assay (outlined below). In this presentation, all soil weights are given on an oven dried basis.

Test Chemicals

The pesticide used was the insecticide endosulfan [(1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebismethylene) sulfite]. Endosulfan was added to test systems either as a technical grade compound (α-β-endosulfan; 95% pure; Chem Service Inc., West Chester, PA, USA) or as the commercially formulated product (Thiodan 4EC; 400 g endosulfan L^{-1}; Chipman Chemicals Ltd., Stoney Creek, Ontario, Canada). Endosulfan has two stereoisomers, α- and β-endosulfan, which are present in Thiodan in an approximate ratio of 70:30 (Martens, 1976). The technical grade endosulfan used contained a similar mixture of the two isomers (as labeled). Stock solutions of technical grade endosulfan were prepared in acetone (pesticide research grade; Caledon Laboratories Ltd., Georgetown, Ontario, Canada), while Thiodan was diluted with glass-distilled water to achieve the same concentrations of active ingredient. All pesticide concentrations are given as parts per million of active ingredient (microgram of endosulfan per gram of oven dry weight of soil). Acetone concentrations are given as percent (v/w).

Nitrification

Nitrification in soil was measured using both a soil perfusion technique and a batch incubation procedure. In both cases ammonium ion was

added to control and endosulfan-treated soil samples, and the accumulation of nitrate ion was followed over time, as outlined below. All experiments were performed at a temperature of 25 ± 1°C.

The soil perfusion technique employed a reperfusion apparatus (after Lees and Quastel, 1946; Parkinson *et al.*, 1971). This consisted of a reservoir, a soil column, and interconnecting glass tubing. The reservoir was constructed from an open-ended, 250-mL, long-stem, glass separatory funnel, stoppered at one end with a rubber stopper containing connections for the soil column, a sampling port, and a vacuum port. The stem was bent upward to facilitate tubing connections to the soil column. The soil column was constructed from 25-mm-outer diameter (OD) glass tubing, 21 cm long, and stoppered at both ends with glass wool and rubber stoppers containing glass tubing connectors. The bottom of the soil column was connected directly to the top of the reservoir and the reservoir outlet was connected to the soil column with 4-mm OD glass tubing containing an aeration port. A vacuum manifold provided the negative pressure required to recirculate the contents of the reservoir.

Each reservoir contained 200 mL of a 50 ppm $NH_4^+ - N$ solution, provided as $(NH_4)_2SO_4$ (reagent grade; Fisher Scientific Ltd., Unionville, Ontario, Canada). The soil column contained enough soil to be equivalent to 50 g oven dry weight, and was sieved to a 2–4-mm mesh size. The soil was treated and mixed prior to addition to the soil columns. Treatments were prepared in replicates of five and each experiment was repeated three times. Treatments included a water blank for the determination of background $NO_3^- - N$ concentrations (no $NH_4^+ - N$ in the reservoir), an untreated control, and treated systems containing 10, 50, 100, 500, and 1000 ppm endosulfan, supplied either as the technical grade chemical or the commercial formulation. Stock solutions were prepared so that the proper concentration of active ingredient was added to the soil in a 0.5 mL volume. With technical endosulfan this yielded a background acetone concentration of 1.0% (v/w). In experiments involving technical endosulfan, acetone was also added to soil in the water blanks and the untreated control systems. One-milliliter samples of perfusate were collected daily and analyzed for $NO_3^- - N$, as outlined below.

With the batch incubation method, fresh soil equivalent to 10 g oven dry weight was sieved to a 2–4-mm mesh size, adjusted to a 60% water holding capacity (calculated to allow for the subsequent addition of aqueous solutions), and added to separate 250-mL Erlenmeyer flasks. One milliliter of a 2000 ppm $NH_4^+ - N$ solution, supplied as $(NH_4)_2SO_4$, was added to the soil in each flask. Treatments were prepared in enough replicates to allow five flasks within each group to have their contents

harvested and assayed at each time interval. Treatments included a water blank (no $NH_4^+ - N$ added), an untreated control, and treated systems containing 10, 100, and 1000 ppm of endosulfan, supplied either as the technical grade chemical or the commercial formulation. Stock solutions were prepared so that the proper concentration of active ingredient was added to the soil in a 0.1-mL volume. Again, this yielded a background level of 1.0% acetone in systems containing technical endosulfan. The flasks were sealed with cork stoppers and any water lost by evaporation was replaced periodically by weight. At various time intervals flasks were sacrificed for $NO_3^- - N$ analysis. Nitrate was extracted by adding 100 mL of glass-distilled water to the flasks, followed by shaking for 1 h on a mechanical shaker. After settling and filtration through Whatman No. 42 filter paper, 1.0-mL samples were removed and analyzed for $NO_3^- - N$, as outlined below. All experiments were repeated three times.

Nitrate-N was quantitated colorimetrically using the phenol disulfonic acid procedure (Bremner, 1965). All chemicals were reagent grade (Fisher Scientific Ltd., Unionville, Ontario, Canada). Absorbance values were converted to $NO_3^- - N$ concentrations (micrograms per milliliter of perfusate or extraction medium) using standard curves prepared daily.

Data Analysis

All nitrate concentrations were recalculated on a soil weight basis (microgram of $NO_3^- - N$ per gram of oven dry weight soil) and data from replicate experiments were combined and plotted against time. Curves were fitted to the data using third-order polynomial regression (Cricket Graph, Cricket Software, Malvern, PA, USA). Day zero values refer to the background levels of nitrate contained in the soil prior to treatment. For each toxicant level, the daily nitrate concentrations were also used to calculate percent inhibition values, based upon activity in control systems. The various daily inhibitions for each toxicant level were combined and averaged to yield an overall inhibition value that was used to estimate EC_{50} values (the toxicant concentration required to cause a 50% inhibition of nitrification). Significant differences at $p = 0.05$ were determined using an analysis of variance procedure followed by a Duncan's multiple range test, or a Student's t – test, where applicable (SAS Statistics Software, SAS Inst. Inc., Cary, NC, USA).

RESULTS

The soils used in the present study were chosen to provide a range of soil types with low pH values. Soils in Atlantic Canada are predominantly

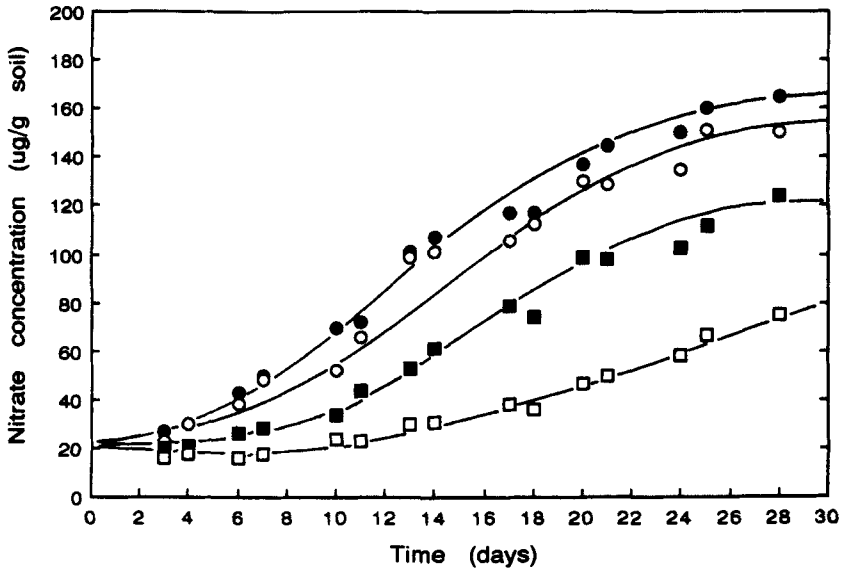

Fig. 1. Effect of acetone on nitrification in a sandy loam soil as measured using soil perfusion. Soil: TSL; (●) control, (○) 0.1% acetone (v/w), (■) 1.0%, (□) 2.0%.

acidic, with coniferous forests dominating uncleared land. Endosulfan was chosen as the test compound because of the lack of data on the toxicity of this insecticide toward nitrification in soil.

Commercially formulated endosulfan (Thiodan) was diluted with water; however, the technical endosulfan had to be dissolved in an organic solvent prior to addition into test systems. Acetone, the solvent of choice in most bioassays involving microorganisms, was used (Majewski *et al.*, 1978). A preliminary study determined the effects of this solvent toward nitrification in soil. Similar toxicity patterns were obtained with all three test soils and representative data for the sandy loam are outlined in Fig. 1. Acetone induced a concentration-dependent increase in the lag phase of nitrification and a reduction in the amount of ammonium ion converted into nitrate during the 30-day incubation (Fig. 1). An acetone level of 1.0%, which was the concentration employed in the present study, caused a 30% inhibition of nitrification. The use of lower solvent levels was impractical, since a minimum volume of test solution was required to evenly distribute the insecticide to the soil samples. Little is known about the effects of acetone toward nitrification (Debnath and Bhattacharya, 1982), but a recent study into the effects of organic solvents on denitrification in soil showed that they can have a

significant effect on this process and can elicit erroneous conclusions regarding the toxicity of the solute (Yeomans and Bremner, 1989). Solvents are capable of interacting with pesticides in bioassays, and can cause synergistic and/or antagonistic responses (Stratton *et al.*, 1982). In the present study acetone was added to the control flasks in all experiments utilizing technical endosulfan and no solvent–pesticide interactions were detected using the method of Stratton *et al.* (1982).

The effects of endosulfan on nitrification in the sandy loam soil, as measured using soil perfusion, are outlined in Fig. 2. Activity in the controls was similar in both series of experiments (Fig. 2A, B). There was a short 1 1/2 to 2-day lag period and a maximum level of between 170 and 180 μg of nitrate/g soil was reached following the 32-day incubation. Endosulfan added as Thiodan (Fig. 2A) was more toxic toward nitrification than was the technical product (Fig. 2B). With Thiodan, a concentration of 500 ppm active ingredient (a.i.) increased the lag period to four d and reduced the nitrate yield to 150 ug/g soil. At 1000 ppm a.i. the lag increased to 8 days with a nitrate yield of around 140 μg/g soil. When data for the entire incubation period were considered, 500 and 1000 ppm of endosulfan, supplied as Thiodan,

TABLE II
Effect of endosulfan on nitrification in soil[a]

Test Parameters[b]	Endosulfan Concentration (Parts Per Million of Active Ingredient)				
	10	50	100	500	1000
TSL					
PER/FOR	7.3 (4.5)^{c}	12.9 (6.5)^{c,d}	16.3 (8.6)^{c}	34.5 (6.7)^{c}	49.6 (9.9)^{c,d}
PER/TEC	−6.4 (7.0)^{d}	5.2 (7.4)^{c}	18.4 (5.4)^{c}	10.1 (5.4)	13.1 (6.7)
BAT/FOR	14.5 (6.6)^{c,e}	ND	25.1 (5.5)^{c,d}	ND	76.7 (6.9)^{e,f}
BAT/TEC	26.2 (5.8)^{f}	ND	29.6 (8.0)^{d}	ND	36.7 (5.2)^{d}
SSL					
PER/FOR	5.1 (6.1)^{c,d}	19.8 (6.3)^{d}	29.6 (5.1)^{d}	62.7 (7.2)	72.9 (6.9)^{e}
PER/TEC	24.2 (6.5)^{f}	32.5 (9.2)	22.1 (9.8)^{c,d}	37.2 (7.4)^{c}	46.4 (5.3)^{c,d}
BAT/FOR	17.4 (4.3)^{e,f}	ND	42.3 (4.2)	ND	86.4 (5.1)^{f}
BAT/TEC	12.9 (4.2)^{c,e}	ND	29.4 (4.4)^{d}	ND	54.4 (6.0)^{c}

[a] Table entries are the mean percent inhibition values, followed by the standard deviation in parentheses, calculated from activity in control systems over the entire incubation period; ND: no data.

[b] Type of incubation: PER—perfusion experiment, BAT—batch incubation; source of endosulfan: FOR—endosulfan as the formulated product Thiodan, TEC—technical grade endosulfan.

[c–f] Values in each column that are followed by the same symbol do not differ significantly at $p = 0.05$.

caused a 34.5 and 49.6% inhibition of nitrification, respectively (Table II). Concentrations of 10, 50, and 100 ppm caused inhibition levels of between 7 and 16% (Table II). Using the data in Table II, an EC_{50} value of 960 ppm a.i. was calculated for endosulfan (Thiodan). In contrast, endosulfan supplied as the technical product had no appreciable effect on the lag phase of nitrification or the nitrate yields when used at concentrations up to 1000 ppm a.i. (Fig. 2B). The calculated percent inhibition values ranged from −6.4 to 18.4% (Table II), and the EC_{50} value was estimated using a regression equation to be >4500 ppm a.i. Technical endosulfan was significantly less toxic than Thiodan at concentrations of 10, 500, and 1000 ppm a.i. (Table II).

Similar results were obtained when nitrification in the sandy loam soil was measured using a batch incubation technique (Fig. 3); however, the data were more variable and nitrification was less efficient. Control systems evidenced a 14–15-day lag period and took 40 days to reach nitrate levels of 170–to 180 μg/g soil (Fig. 3A, B). Fewer concentrations of endosulfan were tested with this system, because of technical limitations caused by the number of flasks and other materials required. Again, endosulfan was more toxic when applied as the formulated product Thiodan (Fig. 3A), although the response was less pronounced than with soil perfusion (Fig. 2). With Thiodan, a concentration of 100 ppm a.i. elicited a 15–16-day lag and reduced nitrate yields to 140 μg/g soil (Fig. 3A). No appreciable activity was apparent at a level of 1000 ppm a.i. until after day 25, when nitrate levels reached a maximum of only 25 μg/g soil. Percent inhibition values of 25.1 and 76.7% were obtained for endosulfan concentrations of 100 and 1000 ppm a.i., respectively (Table II). The calculated ED_{50} for Thiodan was 550 ppm a.i. With technical grade endosulfan a level of 100 ppm a.i. elicited a 16–18-day lag and a maximum nitrate yield of 130 μg/g soil, while 1000 ppm induced a 22–23-day lag and a yield of 120 μg nitrate/g soil (Fig. 3B). The inhibition values ranged from 26 to 37% (Table II) and the EC_{50} was estimated using a regression equation to be >2400 ppm a.i. A comparison of the percent inhibition (Table II) and the EC_{50} data (listed above) obtained from the perfusion and batch incubation systems indicated that endosulfan was generally more toxic when tested using the batch incubation system, regardless of the source of endosulfan used.

The effects of endosulfan on nitrification in the silt loam soil are presented in Figs. 4 and 5. Nitrification in this soil was slower than in the sandy loam. When nitrification was measured using soil perfusion (Fig. 4A), control systems evidenced a lag period of less than 2 days. During the 50-day incubation period, however, the nitrate level reached a maximum of only 70 μg/g soil out of a

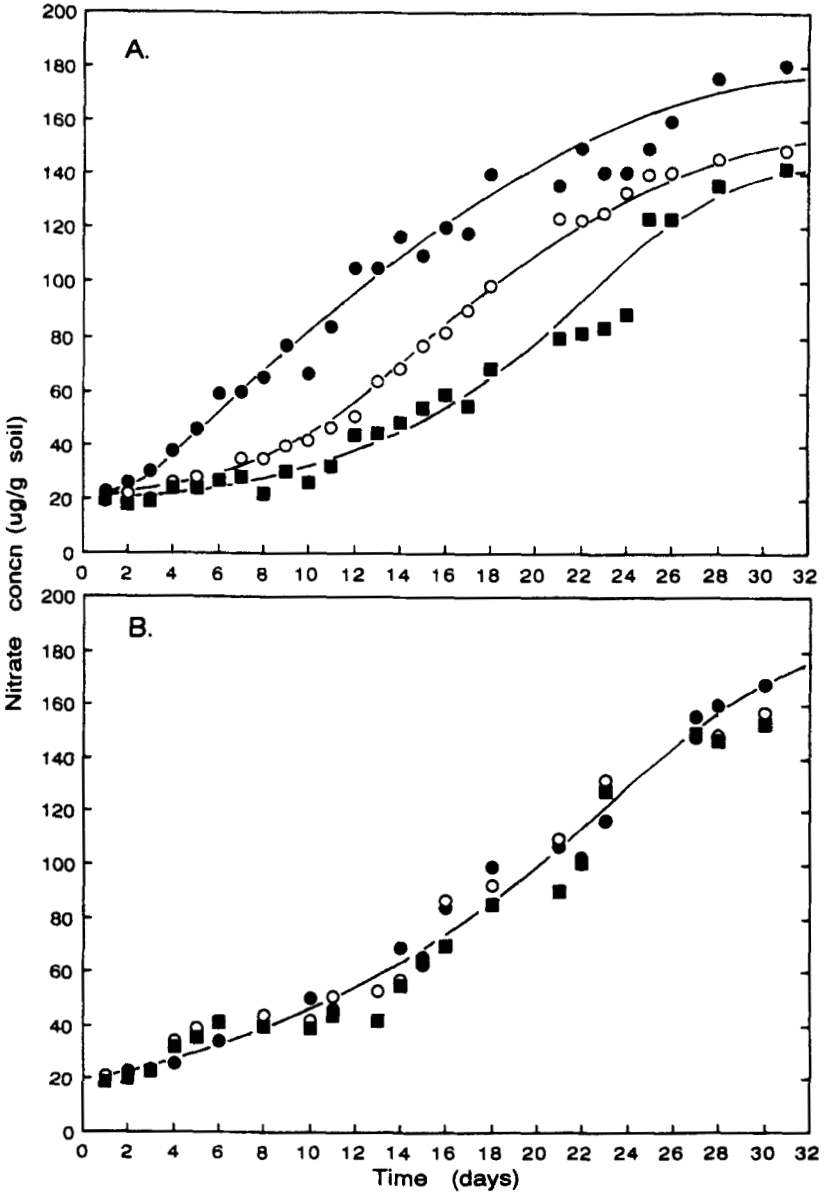

Fig. 2 Effect of endosulfan on nitrification in a sandy loam soil as measured using soil perfusion. (A) Endosulfan as the formulated product Thiodan; (B) technical grade endosulfan; Soil: TSL; (●) control, (○) 500 ppm endosulfan; (■) 1000 ppm.

theoretical 200 μg (based upon the total amount of ammonium ion added to the soil). Thiodan, at concentrations of 100 and 500 ppm endosulfan, caused a slight increase in the lag phase and reduced the amount of nitrate to 60 and 40 μg/g soil, respectively (Fig. 4A). A level of 1000 ppm a.i. increased the lag to 6 days and reduced the yield to 25 μg nitrate/g soil. The corresponding inhibition levels are summarized in Table II. The calculated EC_{50} value for endosulfan, when supplied as the formulated product, was 525 ppm a.i. Data for technical grade endosulfan are less complete. The controls evidenced a longer lag period (up to 10 days), but similar nitrate levels were attained during the 35-day incubation (Fig. 4B). Again, the technical material was less toxic than the formulated product, but only at the higher concentrations tested (Fig. 4, Table II). The EC_{50} of technical endosulfan was calculated as 1130 ppm a.i.

Nitrification in the silt loam soil was also less efficient when measured using the batch incubation method (Fig. 5). Control systems required 65 days to reach a nitrate level of 60 μ/g soil and had lag periods of up to 15 days (Fig. 5A, B). With Thiodan, concentrations of 100 and 1000 ppm a.i. caused an increase in the lag period of up to 40 days and reduced the level of nitrate obtained to 40 and 10 μg/g soil, respectively (Fig. 5A). The corresponding inhibition values are listed in Table II and were used to calculate an EC_{50} value of 390 ppm a.i. With technical grade endosulfan, concentrations of 100 and 1000 ppm a.i. both elicited lag periods lasting from 15 to 20 days and reduced nitrate levels to 40 and 30 μg/g soil, respectively (Fig. 5B). The inhibition values are summarized in Table II and were used to calculate an EC_{50} value of 870 ppm a.i. The formulated product was significantly more toxic than the technical material at the two highest concentrations tested (Table II). Again, endosulfan was generally more toxic toward nitrification when measured using a batch incubation method. When the sandy loam and silt loam soils were compared, inhibition values for any given set of test conditions were generally higher with the latter (Table II).

Activity in the clay loam soil (pH 4.9) was very low. When nitrification was measured using soil perfusion, control systems converted only 5% of the added ammonium ion into nitrate during a 70-day incubation period. No nitrifying activity could be measured with batch incubation systems during a similar period of time. Although activity in perfusion experiments was low, trends in results similar to those reported above were obtained. Endosulfan as the formulated product caused noticeable reductions in activity at concentrations of 500 and 1000 ppm, however, technical endosulfan caused no detectable inhibition at these concentrations. No further comparisons could be made, due to the low activity in these test systems.

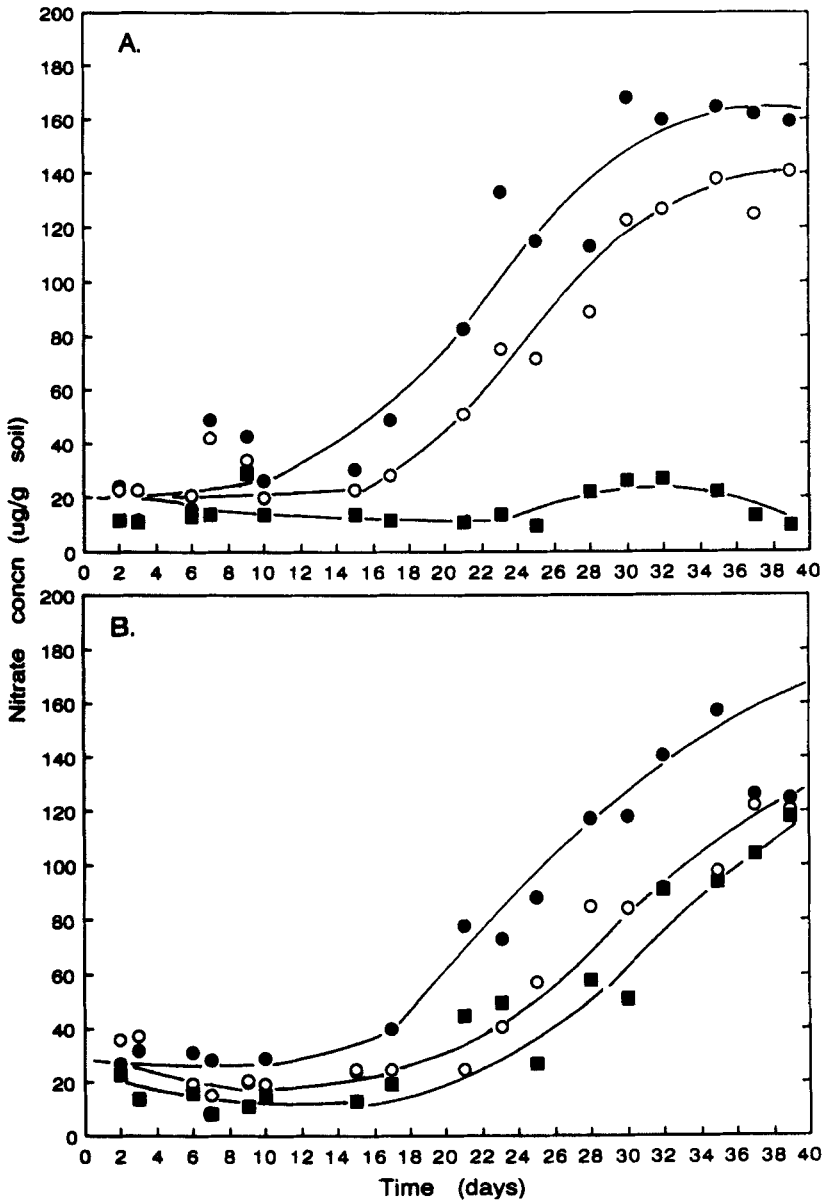

Fig. 3 Effect of endosulfan on nitrification in a sandy loam soil as measured using batch incubation. (A) Endosulfan as the formulated product Thiodan; (B) technical grade endosulfan; Soil: TSL; (●) control, (○) 100 ppm endosulfan, (■) 1000 ppm.

DISCUSSION

Few other data are available on the effects of endosulfan toward nitrification in soil for comparison with results from the present study. Debnath and Bhattacharya (1982) reported that Thiodan concentrations from 0.9 to 4.4 ppm of active ingredient caused a significant inhibition of nitrification in an alluvial soil (pH 7.5), when measured using a batch incubation procedure. Recommended field application rates of Thiodan would give a level around 1.0 ppm of active ingredient if the pesticide was incorporated into the soil (Debnath and Bhattacharya, 1982) and levels considerably higher if exposure was restricted to the soil surface. In the present study the lowest level tested was 10 ppm, which caused up to 26% inhibition of nitrification (Table II). Significant inhibitions may have been evident at lower concentrations, but this can only be speculated upon. One noticeable difference between the present study and that of Debnath and Bhattacharya (1982) is that the latter used air-dried soil while the former employed freshly collected soil. Air drying of soil causes a significant burst in microbial activity upon rewetting (Parkinson *et al.*, 1971), and this may have an influence on the susceptibility of microbial populations to toxicants, although this possibility can only be verified following additional research. As well, the endosulfan concentrations used in the present study are standardized on a soil dry weight basis, while those of Debnath and Bhattacharya (1982) were not. This makes it difficult to compare the data for various concentrations of toxicant.

In the present study, endosulfan concentrations hundreds of times greater than field rate had to be tested in order to obtain a 50% inhibition of activity. High concentrations of endosulfan also induced an increase in the lag phase of nitrification (Figs. 2–5). A similar response has been reported in other studies (reviewed in Reddy *et al.*, 1984), and this is possibly due to a reduction in microbial numbers and the time required for those populations to recover (Reddy *et al.*, 1984). Reviews dealing with the effects of insecticides on microorganisms and microbial activities in soil (Tu and Miles, 1976; Reddy *et al.*, 1984), and on the effects of pesticides toward nitrification (Dhanaraj, 1988; Ray and Sethunathan, 1988), refer to no other endosulfan data. Insecticides, however, usually do not affect nitrifying populations or their activities when used at normal field application rates (Reddy *et al.*, 1984; Dhanaraj, 1988). Where inhibitory effects are noticed they are usually short-lived and nitrification rates recover to control values over time (Reddy *et al.*, 1984; Dhanaraj,1988). This recovery may be due to degradation of the insecticide or its detoxification by nitrifying populations (Reddy *et al.*, 1984).

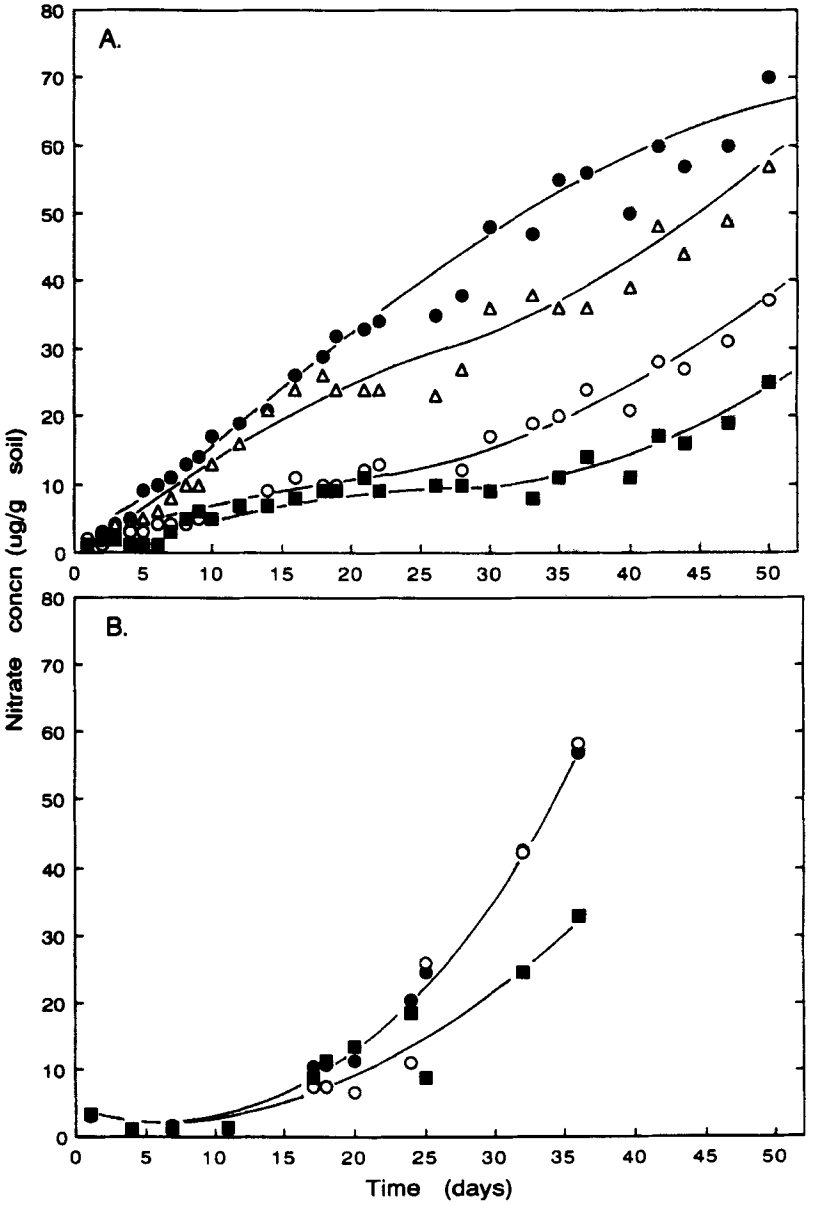

Fig. 4 Effect of endosulfan on nitrification in a silt loam soil as measured using soil perfusion. (A) Endosulfan as the formulated product Thiodan; (B) technical grade endosulfan; Soil: SSL; (●) control, (△) 100 ppm endosulfan, (○) 500 ppm, (■) 1000 ppm.

Recovery is also affected by the temperature, organic matter content, clay content, cation exchange capacity, and pH (Reddy *et al.*, 1984). In the present study recovery was not apparent during the incubation periods employed.

In this study the formulated product Thiodan was more toxic than technical grade endosulfan at equal levels of active ingredient. Other researchers have reported similar results (Reddy *et al.*, 1984). In particular, Kuseske *et al.* (1974) found that the commercial formulations of Baygon and Temik were more toxic than the pure insecticides propoxur and aldicarb, respectively, when tested toward nitrification in soil. The higher toxicity of the formulated material may be due to interaction effects between the various solvents, cosolvents, and other components within the product (Stratton *et al.*, 1982). Insecticide formulations contain adjuvants to enhance the efficacy of the active ingredient. No data are available, however, on the toxicity of pesticide adjuvants toward nitrification in soil. All ingredients contained in commercial preparations are xenobiotics and therefore have the potential to be toxic toward nitrification in soil.

As noted above, endosulfan was more toxic toward nitrification when measured using a batch incubation method. Few other data of this nature are available for comparison purposes. Both soil perfusion and batch incubation procedures are classified as closed systems (Prosser and Cox, 1982). Metabolic products accumulated during the experiment and substrate levels are continually changing. It is impossible to maintain a constant microbial growth rate in closed systems (Prosser and Cox, 1982). The batch incubation system is more heterogeneous, since uniform mixing of water and ammonium ion is difficult. In the perfusion system a homogeneous distribution of the soil solution and the maintenance of both a constant moisture and oxygen content is possible (Lees and Quastel, 1946). This encourages a reduced lag period and a more rapid turnover of ammonium ion. This difference in nitrification patterns was noted during the present study (Figs. 2–5). Perfusion systems also suffer from several disadvantages, including the necessity for near waterlogged conditions and the loss of perfusate volume during sampling (Lees and Quastel, 1946). Based upon the data presented here, the use of a batch incubation system will give higher toxicity estimates for test compounds than will a perfusion system. This may be desirable when screening xenobiotics for their potential environmental impact. If more accurate estimates are required, a perfusion system could be used.

There was also a significant difference in the nitrification pattern obtained with the three acidic soils. Reasons for this can only be speculated upon. Nitrification can either be autotrophic or heterotrophic, and

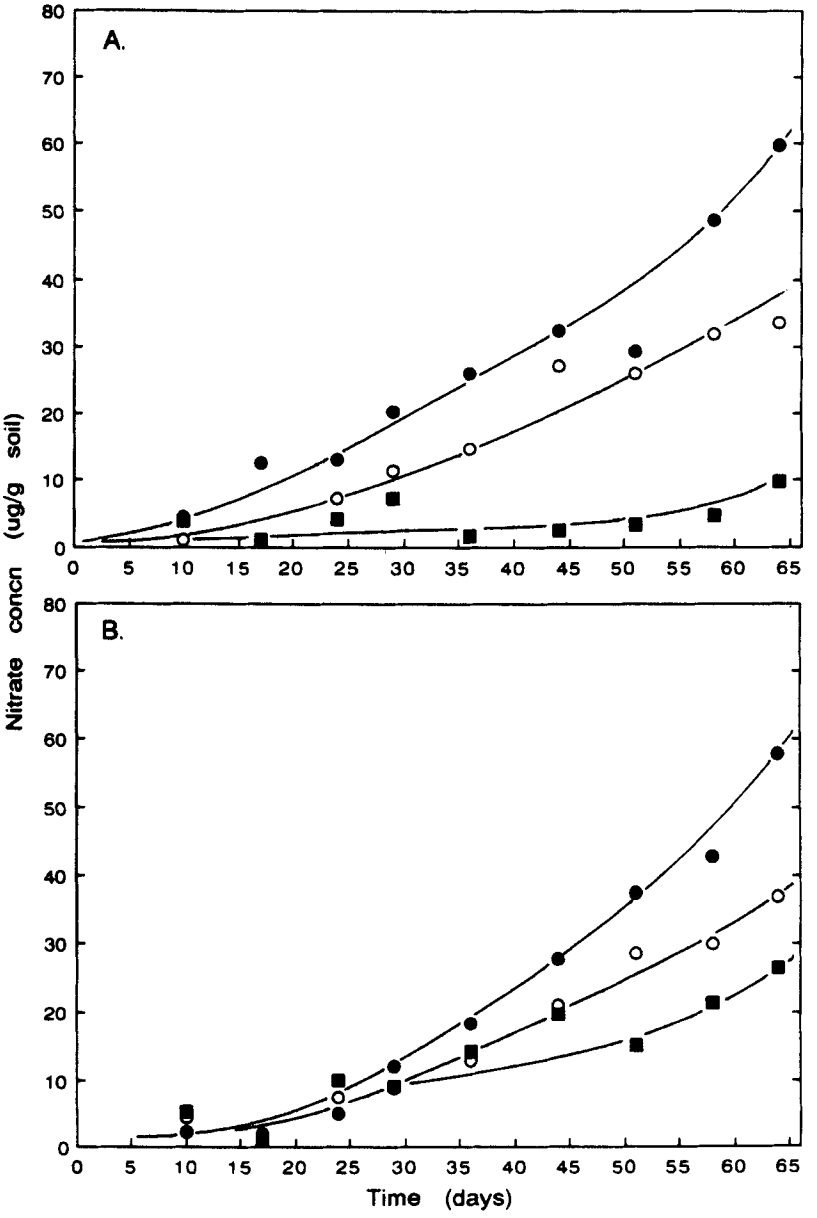

Fig. 5 Effect of endosulfan on nitrification in a silt loam soil as measured using batch incubation. (A) Endosulfan as the formulated product Thiodan; (B) technical grade endosulfan; Soil: SSL; (●) control, (○) 100ppm endosulfan, (■) 1000 ppm.

soil pH is a particularly important factor affecting both. The most favorable range for autotrophic nitrification is pH 7.0–9.0, with inhibition evident below pH 6.0 (Prosser and Cox, 1982; Haynes, 1986). The lower pH limit for autotrophic nitrification is around 4.5 (Haynes, 1986). Heterotrophic nitrification is less commonly encountered but is often important at pH values unfavorable for the growth of autotrophic nitrifiers (Prosser and Cox, 1982). Nitrification is still evident at pH 5.0 in many soils and the pH optima for nitrification in some acid soils may be as low as 4.5 (Haynes, 1986). This has often been shown to be due to heterotrophic nitrification (Lang and Jagnow, 1986), although autotrophic nitrifiers have been isolated from soil with pH values as low as 4.3 (Hankinson and Schmidt, 1988). Most research on nitrification in acid soils has concentrated on forest systems (e.g., Adams, 1986; Hankinson and Schmidt, 1988) and data for agricultural soils are limited. In a recent study of nitrification in Atlantic coastal plain soils appreciable nitrifying activity was observed in some soils with a pH of 4.5–4.6 (Weier and Gilliam, 1986). However, other similar soils evidenced no activity. It was suggested that the level of organic matter was an important factor affecting nitrification in acid soils (Weier and Gilliam, 1986). No attempt was made in the present study to determine the extent of heterotrophic nitrification. Differences between test soils were probably due to a number of factors, including those discussed above.

CONCLUSIONS

The data outlined here document the need for further comparative studies on the measurement of nitrification in pesticide bioassays. Nitrification is routinely quantitated using either a perfusion or a batch incubation method. In the present study there were significant differences in the toxicity data obtained by these two procedures. Bioassay methodology must be standardized in order to accommodate the comparison of data obtained from different laboratories. Otherwise, conclusions drawn regarding the environmental impact of pollutants are open to question. With reference to nitrification, a batch incubation system will probably be more sensitive to the xenobiotics being tested. This may be desirable in screening studies designed to identify compounds requiring further investigation. A perfusion system is less sensitive to pesticides but may yield more accurate toxicity data.

A significant difference was also obtained between the toxicity of endosulfan supplied as the formulated product Thiodan and the unformulated technical grade insecticide. This indicates that both commercial preparations and the pure active ingredients must be evaluated

for toxic effects toward nontarget organsims and processes in soil. Endosulfan caused a significant inhibition of nitrification at concentrations as low as 10 ppm, but levels considerably higher than recommended field rates were required for substantial effects. This insecticide should have no long-term deleterious effects on soil nitrification when used at recommended application rates, given the test conditions employed in the present study.

The difference in sensitivity between the three test soils emphasize the need to repeat toxicity bioassays under a number of test conditions. This will ensure a more accurate estimate of potential environment impact. With nitrification, more data are needed on the effects of pesticides toward this process in low pH agricultural and forest soils and soils stressed by other limiting factors.

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