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Effect of six triorganotin(IV) compounds on nitrification and ammonification in soil

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The effects of six triorganotin(IV) compounds and of Thiram on nitrification and ammonification in soil were investigated. Low concentrations of up to 50 $\mu g\ g^{-1}$ of the triorganotin(IV) compounds enhanced nitrate-nitrogen (NO_3^--N) production in soil. Except for diphenylbutyltin bromide, which inhibited nitrification at 250 $\mu g\ g^{-1}$, the other triorganotin(IV) compounds were inhibitory at concentrations of 100 $\mu g\ g^{-1}$ to less than 250 $\mu g\ g^{-1}$. At 10 $\mu g\ g^{-1}$, only triphenyltin acetate was less inhibitory towards nitrification compared with Thiram. At 250 $\mu g\ g^{-1}$, Thiram exerted a strongly persistent inhibitory effect towards nitrification. The NO_3^--N level recorded 28 days after application was only 0.10 $\mu g\ g^{-1}$ soil. With the triorganotin compounds NO_3^--N levels of 7.05–12.06 $\mu g\ g^{-1}$ soil were recorded 28 days following their application. The deleterious effects of the triorganotin(IV) compounds were less persistent and recovery of nitrification was evident seven days after application. Low concentrations of Thiram and triorganotin(IV) compounds inhibited ammonification, whereas higher concentrations enhanced ammonification. Complete inhibition of ammonification was attained 21–28 days after application of Thiram at 50 $\mu g\ g^{-1}$. On the other hand, with the triorganotin(IV) compounds, except for diphenylbutyltin bromide at 10–50 $\mu g\ g^{-1}$, ammonification persisted at all concentrations 28 days after application.

Keywords: Organotins, soil nitrification, soil ammonification

INTRODUCTION

Compared with other soil microbial activities, nitrification and ammonification in soil and the effects of pesticides on the two processes, both in the laboratory and in the field, have been fairly well studied. The effects of fungicides on nitrification and ammonification in soil have been reviewed by Domsch,[1,2] Prasad, Rajale and Lakhdive,[3] Wainwright and Pugh,[4] and Anderson.[5]

Fungicides may severely suppress nitrification in soil. Audus[6] showed strong suppression followed by slow recovery by the fungicides Ferbam (28), Maneb (25), Nabam (60), Thiram (60) and Zineb (17), the numerals given in parentheses being recovery time in days.

Vapam at 224 kg ha^{-1} (or at 560 lb ha^{-1}) inhibited nitrification for 48 weeks while higher rates up to 448 and 596 kg ha^{-1} completely prevented nitrification.[7,8] Agnihotri[9] found that Captan inhibited nitrification for up to two to three weeks, depending on the concentration of the compound applied. Wainwright and Pugh[4] showed that Captan, Thiram and the organomercury compound, Verdasan, were effective inhibitors of nitrification. Barnes *et al.*[10] showed that, under laboratory conditions, triphenyltin acetate at 10 $\mu g\ g^{-1}$ soil did not adversely affect soil nitrification over a period of 120 h. From the foregoing as well as other studies reported by Wainwright and Pugh,[11] Mahmoud *et al.*,[12] Dubey,[13] Dubey and Rodriguez,[14] Wainwright and Sowden,[15] Hofer *et al.*,[16] van Faassen,[17] Casseley and Broadbent,[18] and Agnihotri,[19] it is apparent that fungicides can be potent inhibitors of soil nitrification.

While retardation of nitrification may be harmful for some crops such as lettuce, it can result in improved crop growth and yield in other cases.[20–22] Pugh, Williams and Wainwright[23] suggested that the improved growth could be due to the conservation of

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soil nitrogen in the ammonium (NH_4^+) form which is held in cation exchange and not easily leached from the soil. If the soil nitrogen is in the nitrate (NO_3^-) form, however, then it is easily leached from the soil, leading to groundwater pollution.[24]

Due probably to decreased fungal growth and competition, and also to increased bacterial ammonification of the protein of dead fungal biomass, ammonification is generally favoured by fungicide treatment of soil. With increasing concentrations of Verdasan, Thiram and Captan, for example, Wainwright and Pugh[4] showed increased ammonification in soil (pH 6.7, organic carbon 6.9%). Similarly in soil of pH 5.3, Wainwright and Pugh[11] reported enhanced ammonification with Benomyl, Captan, Quintozene or Thiram treatment.

Saxena,[25] in a review of the toxicology and biomedical applications of organotin compounds, cited a considerable number of studies reporting the fungicidal, insecticidal, arachnicidal, herbicidal, molluscicidal and bactericidal activities of known and candidate organotin compounds. All the studies cited focused on the target pathogen, pest or weed. Kreutzer,[26] in his review, however, concludes that while there is a need for more efficient and specific soil fungicides, perhaps more important is the need for more information on the complex matrix which is treated, i.e. the soil and its microflora. Because many fungicidal compounds are not target-specific, a wide variety of non-pathogenic saprophytic fungi and other microorganisms may be adversely affected, leading to environmentally undesirable consequences.

The aim of the present study was to investigate the effects of six triorganotin(IV) compounds on nitrification and ammonification in ammonium sulphate [$(NH_4)_2$ SO_4]-amended soil. The compounds were selected on the basis of their promising antifungal activities.[27,28] Thiram 50WP was used for comparison while untreated soil with and without ammonium sulphate served as controls.

MATERIALS AND METHODS

Physicochemical properties of the soil

Air-dried and sieved (<2 mm) soil was used. The soil used in the study was obtained from field plots planted with pepper (*Piper nigrum*) cuttings. The soil was classified as a sandy loam comprising of 97% sand (that fraction with particles > 0.02 mm diam.) and 3% silt (that fraction with particles <0.02 mm diam.). The water holding capacity (WHC) of the soil as a whole was 30%, the total nitrogen content 0.14% and the total organic carbon content 21.7%.

Fungicidal compounds

The following triorganotin(IV) compounds were assessed for their effects on nitrification/ammonification in soil:

(i) diphenylbutyltin bromide;
(ii) *p*-tolyldiphenyltin acetate;
(iii) triphenyltin acetate;
(iv) triphenyltin chloride·triphenylphosphine oxide;
(v) triphenyltin indole-3-acetate;
(vi) 2-ethylamino-4-triphenylstannoxy-5-n-butyl-6-methylpyrimidine.

Thiram used was a powder with active ingredient (a.i.) 50% w/w tetramethylthiuram disulphide.

Soil treatment

Fresh soil collected immediately before the experimental run was air-dried overnight and sieved (<2 mm). Excessive air-drying was avoided as this has been reported to lead to increased mineralization of both carbon and nitrogen.[29]

Ammonium sulphate solution (1 cm^3) containing 200 μg ammonium-nitrogen (NH_4^+-N) was added to 200 g of air-dry soil. Stock 1000 $\mu g\ cm^{-3}$ concentrations of triorganotin(IV) compounds and of Thiram 50WP were prepared by dissolving 50 mg (a.i.) of the compound in 5 cm^3 of A.R.-grade acetone and making up to 50 cm^3 with sterile distilled water. Varying volumes of this stock were added to the soil. The soils were then brought to two-thirds their water-holding capacity (WHC) with sterile distilled water to give final concentrations of 10, 25, 50 and 100 $\mu g\ g^{-1}$ soil of each of the triorganotin(IV) compounds and of Thiram 50WP. A further concentration of 250 $\mu g\ g^{-1}$ soil of the above compounds was prepared by dissolving 50 mg (a.i.) of the compounds in 5 cm^3 of acetone and by adding enough sterile distilled water to give two-thirds of WHC when added to the soil. Controls with and without ammonium sulphate were included.

Samples (50 g) of soils were then weighed into

sterile plastic bags, and 0.2 g of calcium hydroxide [$Ca(OH)_2$] was thoroughly mixed with the soils in order to neutralize the acid produced by nitrification. The bags were tied with rubber bands leaving a small hole in the centre. The bags were arranged in a randomized complete block design and incubated at 27 ± 2°C in the dark. The moisture content of the soils was maintained at two-thirds of WHC throughout the experiment by adding sterile distilled water. Four replicates per treatment were included.

The amounts of nitrate- and ammonium-nitrogen were determined at 1, 7, 14, 21, and 28 days after treatment for each replicate.

Determination of nitrate-nitrogen

Nitrate-nitrogen was determined spectrophotometrically using the technique of Clarke and Jennings[30] with modifications after Sims and Jackson.[31]

Soil (5 g) was shaken for 15 min with 25 cm^3 of 0.2% $Ca(OH)_2$ solution in an orbital shaker moving at 275 rpm. The suspension was allowed to settle, and the supernatant was filtered through a Whatman No. 1 filter paper. A volume of 7 cm^3 of 0.01% (w/w) chromotropic acid solution (4,5-dihydroxynaphthalene-2,7-disulphonic acid; CTA) from a burette was added to 3 cm^3 of the clear filtrate in Pyrex test-tubes (Sims and Jackson[31]) and vortexed. The tubes were immediately cooled to below 40°C in running tap-water and then incubated for 20 min in a water bath held at 40°C. At the end of this period, the contents were vortexed again and cooled below 40°C. The intensity of the yellow CTA–nitrate complex was determined at 430 nm in a spectrophotometer using silica cells of path-length 1 cm and slit-width 2 mm; 0.2% $Ca(OH)_2$ solution was used as blank. Concentrations of nitrate-nitrogen present were then calculated by reference to a calibration plot.

Determination of ammonium-nitrogen

Soil (2 g) was shaken with 20 cm^3 of 1.5 mol dm^{-3} potassium chloride (KCl) solution for 1 h in an orbital shaker at 275 rpm. The suspension was allowed to settle and the supernatant was filtered through a Whatman No. 1 filter paper. EDTA (1 cm^3, 6%) was added to 2 cm^3 of filtrate to chelate any metal ions;[32] 7 cm^3 of distilled water, 5 cm^3 of sodium phenolate (*ca.* 40% w/v solution) and 3 cm^3 of sodium hypochlorite solution (5% available Cl) were then added from fast-flowing burettes. Fresh reagents were made up at concentrations suggested by Parkinson *et al.*[33] The resulting solution was thoroughly mixed and incubated at 25°C in a water bath for 20 min. Tetlow and Wilson[32] had shown the reaction involved to be photosensitive; hence the analysis was performed in 100 cm^3 Erlenmeyer flasks covered with aluminium foil. After incubation the solutions were made up to 50 cm^3 with distilled water and mixed again. The intensity of the Indophenol Blue–ammonium complex was determined at 630 nm in a spectrophotometer using silica cells of path-length 1 cm and slit-width 2 mm. 1.5 mol dm^{-3} potassium chloride solution was used as blank. Concentrations of ammonium-nitrogen present were then calculated by reference to a calibration plot.

RESULTS

Effect of triorganotin(IV) compounds on nitrification in soil

The effects of Thiram and the triorganotin(IV) compounds on nitrification are given in Figs 1–7. The levels of nitrate-nitrogen formed in control soils with and without ammonium sulphate supplementation were similar at any one sampling occasion during the period of study. Consequently fungicide-treated and control soil with ammonium sulphate supplementation alone are subsequently compared.

Compared with the control, concentrations of Thiram of up to 50 $\mu g\ g^{-1}$ enhanced nitrification, especially between 14 and 28 days after application (Fig. 1). At 250 $\mu g\ g^{-1}$, nitrification was significantly ($P = 0.05$) suppressed and was often only just barely detectable even after 28 days. With Thiram at 100 $\mu g\ g^{-1}$, nitrification recovered on day 28 after a period of inhibition from day 1 to day 21. The effects of Thiram at all concentrations were most deleterious up to seven days. The gradual build-up in nitrification subsequently could be an indication of the gradual decline in fungitoxicity of Thiram.

Generally, low concentrations of up to 50 $\mu g\ g^{-1}$ of the triorganotin(IV) compounds enhanced nitrate-nitrogen production compared with control (Figs 2–7). High concentrations of 100–250 $\mu g\ g^{-1}$ of the triorganotin(IV) compounds, however, inhibited nitrate-nitrogen production except in the case of diphenylbutyltin bromide, where nitrification was inhibited only at 250 $\mu g\ g^{-1}$. With all six

Key to Figs 1–14

- Control
- 10.0 μg g^{-1} soil
- 25.0 μg g^{-1} soil
- 50.0 μg g^{-1} soil
- 100.0 μg g^{-1} soil
- 250.0 μg g^{-1} soil

* Significantly different from control at $P = 0.05$

Standard error of the mean (SE)

0.85 μg nitrate-nitrogen g^{-1} soil (Figs 1–7)
0.34 μg ammonium-nitrogen g^{-1} soil (Figs. 8–14)

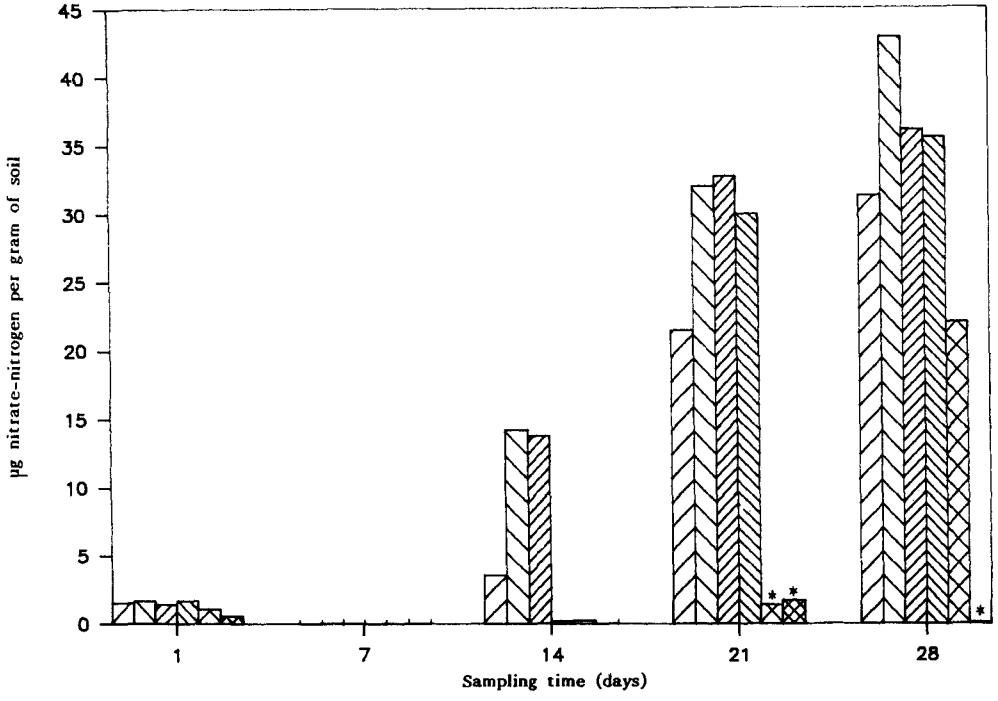

Figure 1 Effect of Thiram on nitrification in soil

triorganotin(IV) compounds, the levels of nitrification were generally lower on day 7 than on all other sampling occasions. Nitrate-nitrogen production was highest on day 28 for all concentrations of the six triorganotin(IV) compounds as well as of Thiram below 250 μg g^{-1}. An initial period of low nitrate-nitrogen production from day 1 to day 7 was followed by a period of high nitrate-nitrogen production from day 14 to day 28 for all the triorganotin(IV) compounds except triphenyltin indole-3-acetate (Fig. 6) and 2-ethylamino-4-triphenyl-stannoxy-5-n-butyl-6-methylpyrimidine (Fig. 7) where the initial period of low nitrate-nitrogen production was extended from day 1 to day 14 indicating a more deleterious effect of these two compounds compared with triphenyltin acetate (Fig. 2), triphenyltin chloride·triphenylphosphine oxide (Fig. 3), diphenylbutyltin bromide (Fig. 4) and *p*-tolyldiphenyltin acetate (Fig. 5). Furthermore, nitrification was

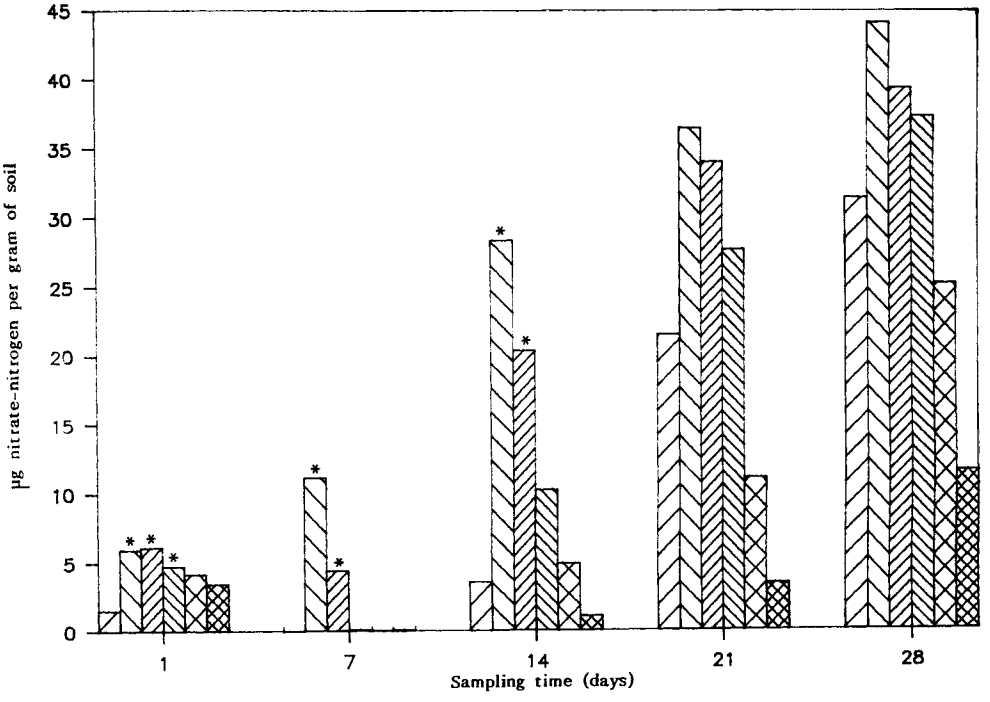

Figure 2 Effect of triphenyltin acetate on nitrification in soil.

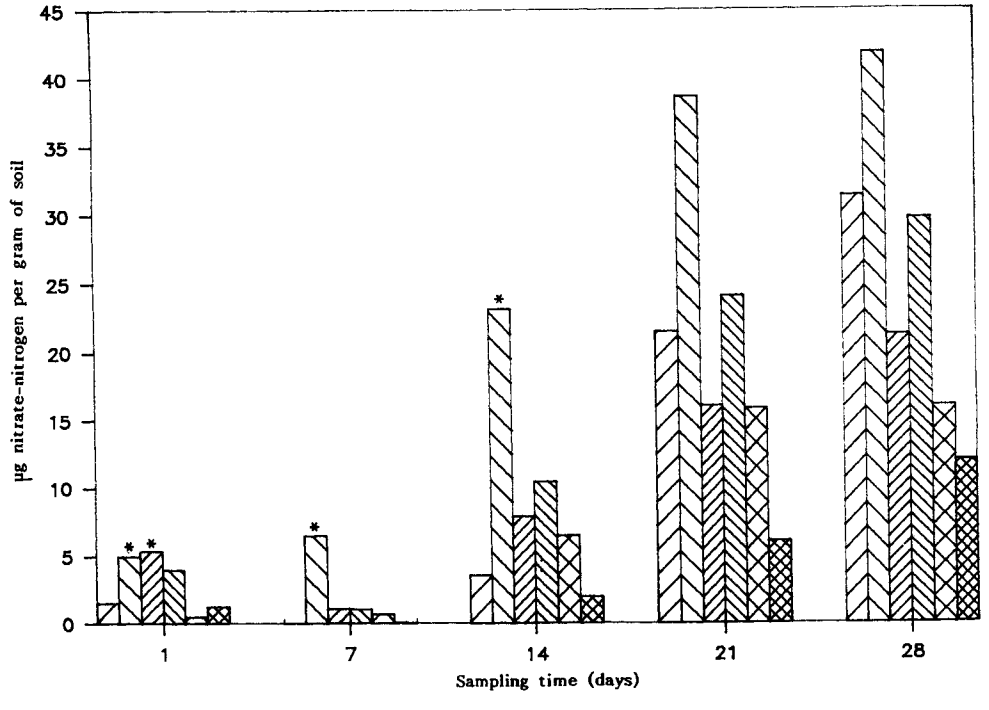

Figure 3 Effect of triphenyltin chloride·triphenylphosphine oxide on nitrification in soil.

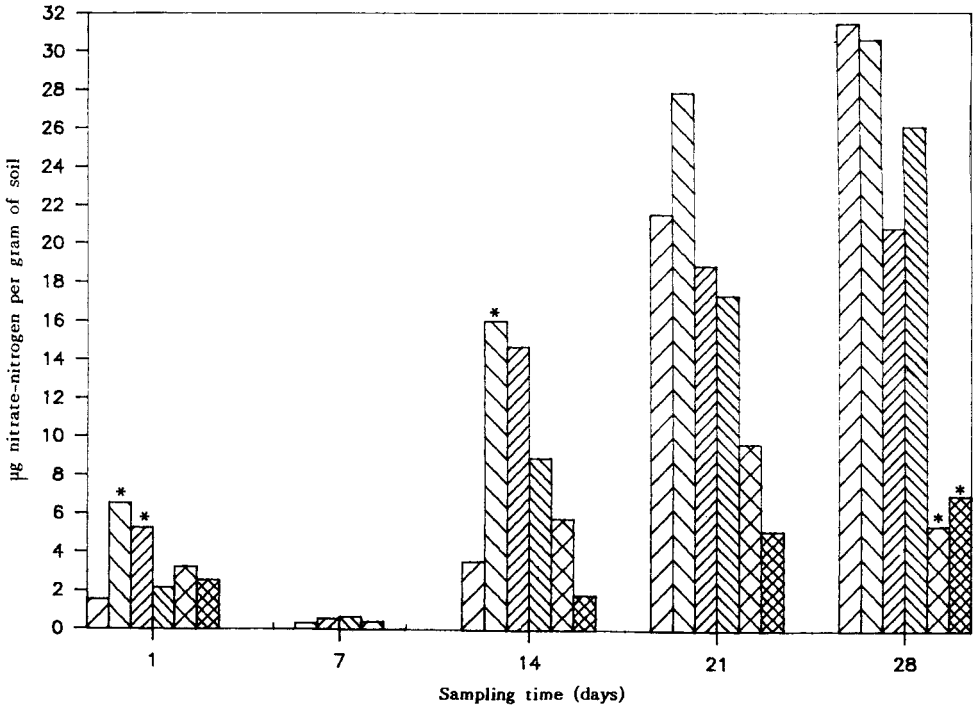

Figure 4 Effect of diphenylbutyltin bromide on nitrification in soil.

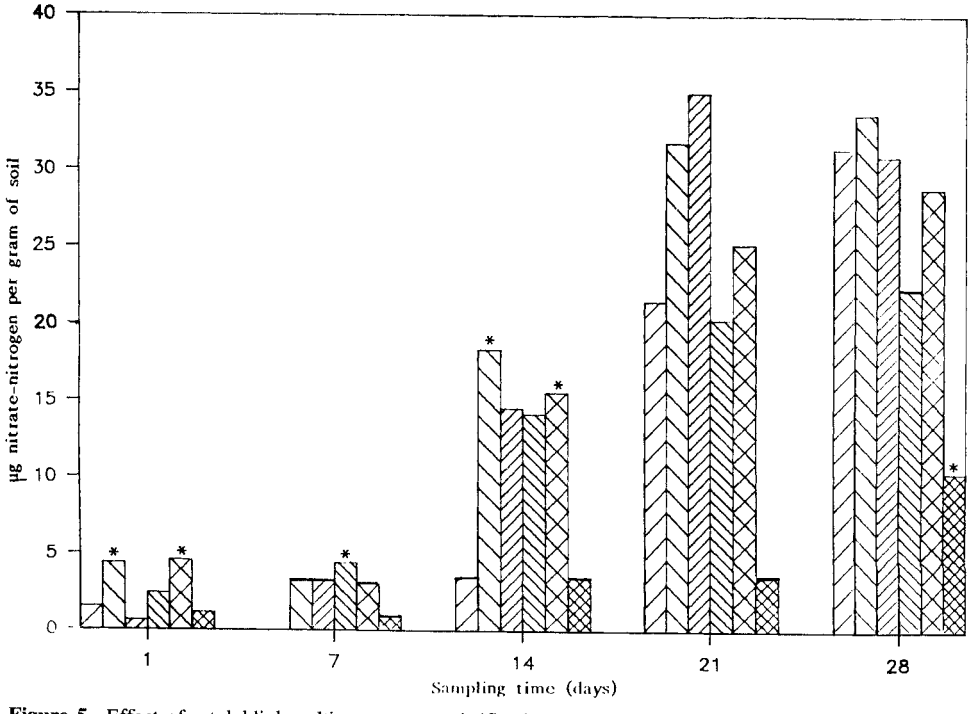

Figure 5 Effect of *p*-tolyldiphenyltin acetate on nitrification in soil.

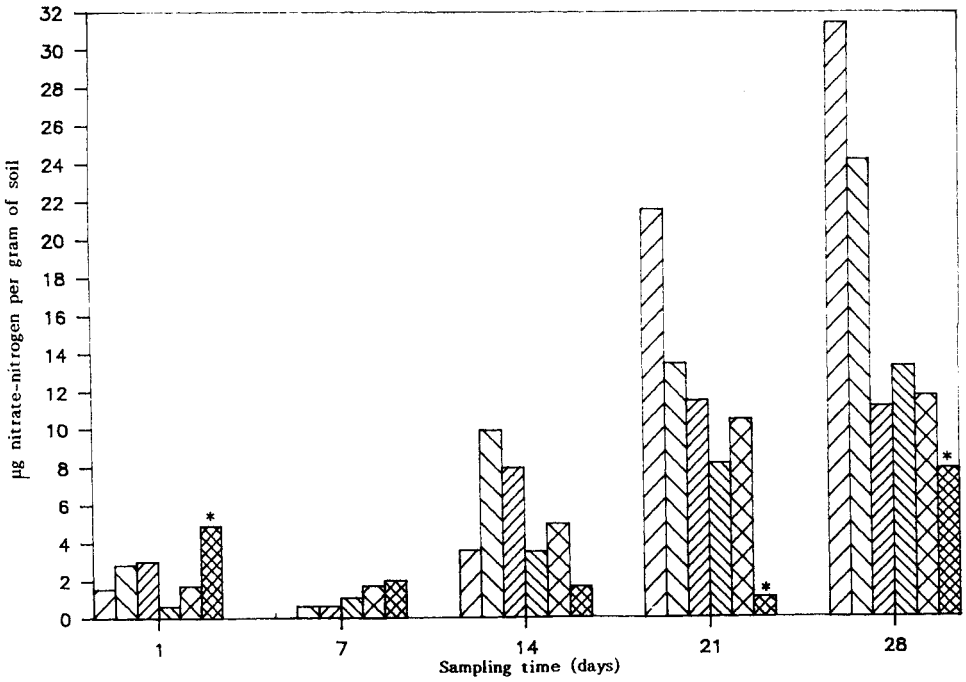

Figure 6 Effect of triphenyltin indole-3-acetate on nitrification in soil.

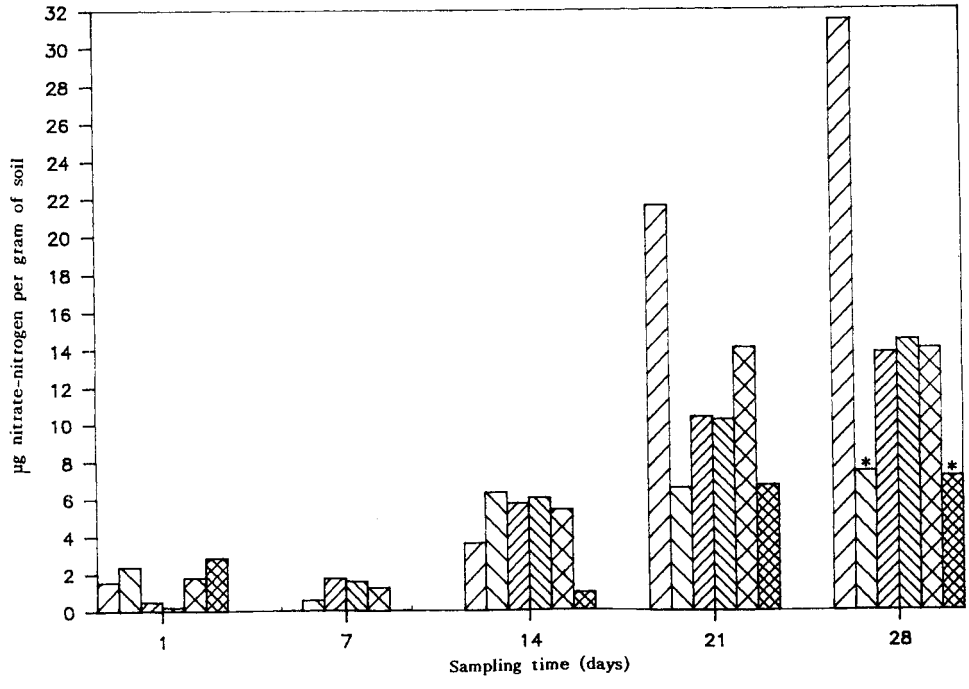

Figure 7 Effect of 2-ethylamino-4-triphenylstannoxy-5-n-butyl-6-methylpyrimidine on nitrification in soil.

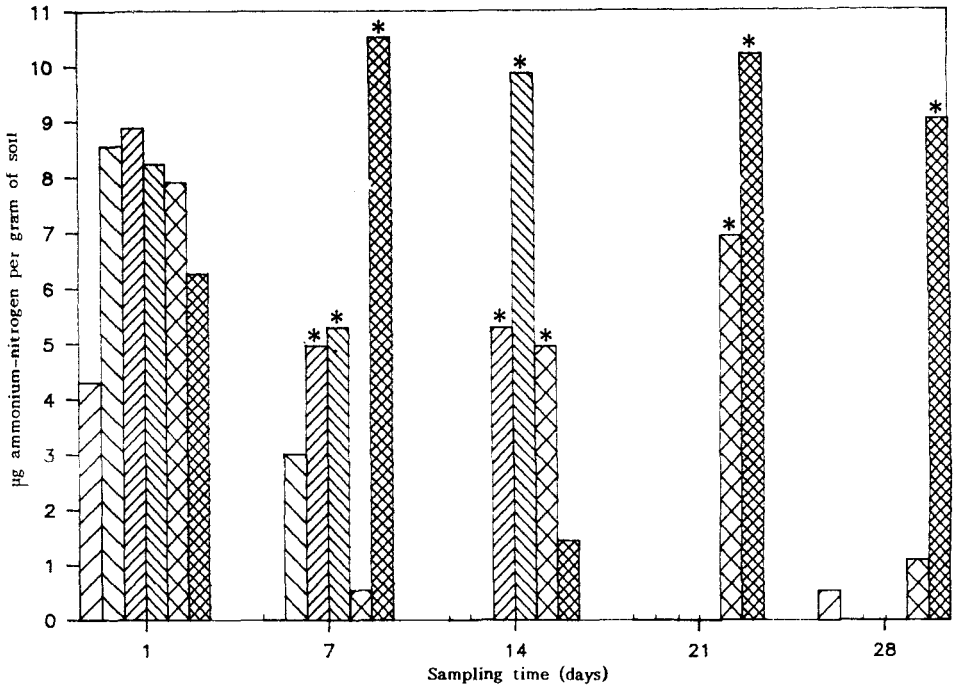

Figure 8 Effect of Thiram on ammonification in soil.

generally low with values ranging from 0 to 24.18 μg nitrate-nitrogen g^{-1} of soil for triphenyltin indole-3-acetate and 2-ethylamino-4-triphenylstannoxy-5-n-butyl-6-methylpyrimidine. For triphenyltin acetate, triphenyltin chloride·triphenylphosphine oxide, diphenylbutyltin bromide and *p*-tolyldiphenyltin acetate, however, the values ranged from 0 to 44.09 μg nitrate-nitrogen g^{-1} of soil.

The deleterious effects of the triorganotin(IV) compounds and Thiram at 10 μg g^{-1} on nitrification are in the order:

> 2-ethylamino-4-triphenylstannoxy-5-n-butyl-6-methylpyrimidine > triphenyltin indole-3-acetate > triphenyltinchloride·triphenylphosphine oxide > diphenylbutyltin bromide > *p*-tolylphenyltin acetate > Thiram > triphenyltin acetate.

If, however, recovery of nitrate-nitrogen produced at a concentration of 250 μg g^{-1} is considered, the triorganotin(IV) compounds are less deleterious to soil-nitrifying micro-organisms than Thiram, indicating the prolonged persistence of the fungitoxicity of Thiram at 250 μg g^{-1} in soil. On day 28, nitrate-nitrogen production at 250 μg g^{-1} of Thiram was only 0.10 μg nitrate-nitrogen g^{-1} of soil, whereas for the triorganotin(IV) compounds, the values ranged from 7.05 to 12.06 μg nitrate-nitrogen g^{-1} of soil. Furthermore, on day 7 only two triorganotin(IV) compounds, namely triphenyltin chloride·triphenylphosphine oxide and 2-ethylamino-4-triphenylstannoxy-5-n-butyl-6-methylpyrimidine, produced deleterious effects similar to that of Thiram where nitrification was completely suppressed.

Analysis of variance for nitrification indicated significant differences ($P = 0.05$) in nitrification between compounds, between sampling time in days and for the interaction between compound and sampling time in days.

Effect of triorganotin(IV) compounds on ammonification in soil

The effects of Thiram and the triorganotin(IV) compounds on ammonification are given in Figs 8–14. The levels of ammonium-nitrogen formed in control soils with and without ammonium sulphate supplementation were similar at any one sampling occasion during the period of study. For purposes of comparison with Thiram- or triorganotin(IV)-treated soils which were

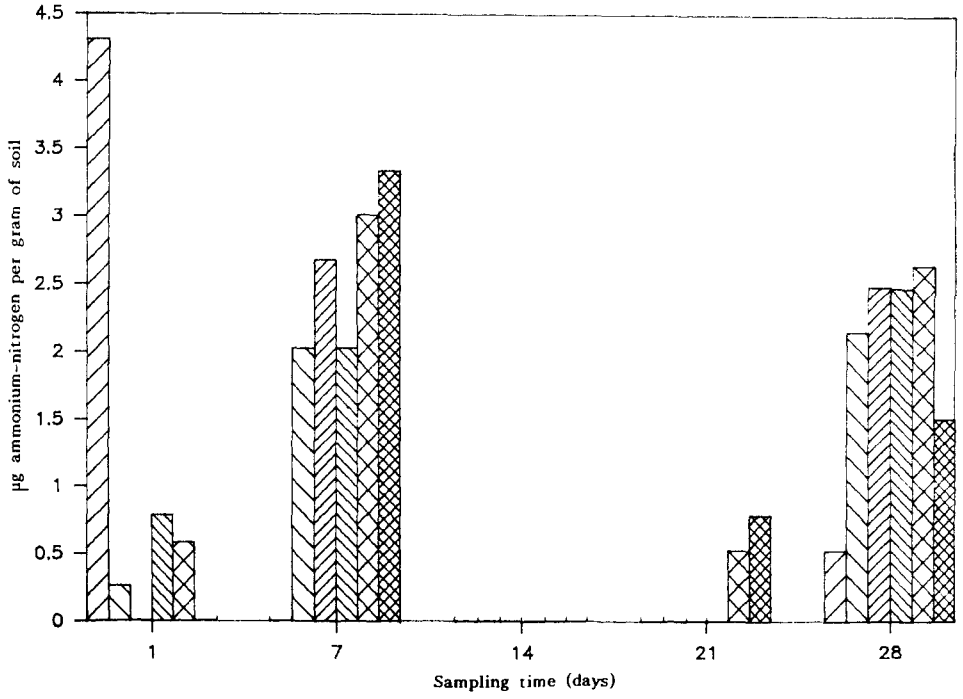

Figure 9 Effect of triphenyltin acetate on ammonification in soil.

also supplemented with ammonium sulphate, control soil with ammonium sulphate supplementation alone was taken.

In Thiram-treated soils, ammonification was enhanced at all concentrations on day 1, followed by a gradual decrease at low concentrations up to 50 $\mu g\ g^{-1}$ with incubation time (Fig. 8). At high concentrations of 100 and 250 $\mu g\ g^{-1}$ of Thiram, however, ammonification fluctuated between 1.08 and 7.91 μg ammonium-nitrogen g^{-1} of soil at 100 $\mu g\ g^{-1}$ and between 1.44 and 10.20 μg ammonium-nitrogen g^{-1} of soil at 250 $\mu g\ g^{-1}$ over the incubation period. Ammonification was always higher than control on all sampling occasions except on day 28 at concentrations of 10, 25 and 50 $\mu g\ g^{-1}$ of Thiram.

Ammonium-nitrogen production was highest on day 1 at all concentrations of the triorganotin(IV) compounds except triphenyltin acetate where the highest ammonium-nitrogen production was recorded on day 7. Ammonification then declined steeply to barely detectable levels of ammonium-nitrogen production until day 14 for triphenyltin acetate (Fig. 9), *p*-tolyldiphenyltin acetate (Fig. 10), triphenyltin chloride·triphenylphosphine oxide (Fig. 11) and diphenylbutyltin bromide (Fig. 12) and was followed by increases in ammonium-nitrogen production indicating the declining persistence of toxic effects of the compounds on ammonifying micro-organisms. In the case of triphenyltin indole-3-acetate (Fig. 13) and 2-ethylamino-4-triphenylstannoxy-5-n-butyl-6-methylpyrimidine (Fig. 14), ammonification declined steeply until day 21 followed by increases in ammonium-nitrogen production, indicating a slightly longer persistence of toxic effects on ammonifying micro-organisms compared with the other triorganotins.

Generally, low concentrations of Thiram and triorganotin(IV) compounds inhibited ammonification whereas high concentrations enhanced it. Ammonification was completely inhibited at concentrations up to 50 $\mu g\ g^{-1}$ of Thiram on days 21 and 28. With the triorganotin(IV) compounds, however, ammonification persisted at all concentrations on day 28 except with diphenylbutyltin bromide at 10 and 50 $\mu g\ g^{-1}$.

Analysis of variance for ammonification showed that the effects of compounds, concentrations and sampling times in days resulted in significant ($P = 0.05$) differences in ammonification rates. The interaction factors, compound and concentration, compound and sampling time, and concentration and sampling time also caused a significant ($P = 0.05$) difference in ammonification.

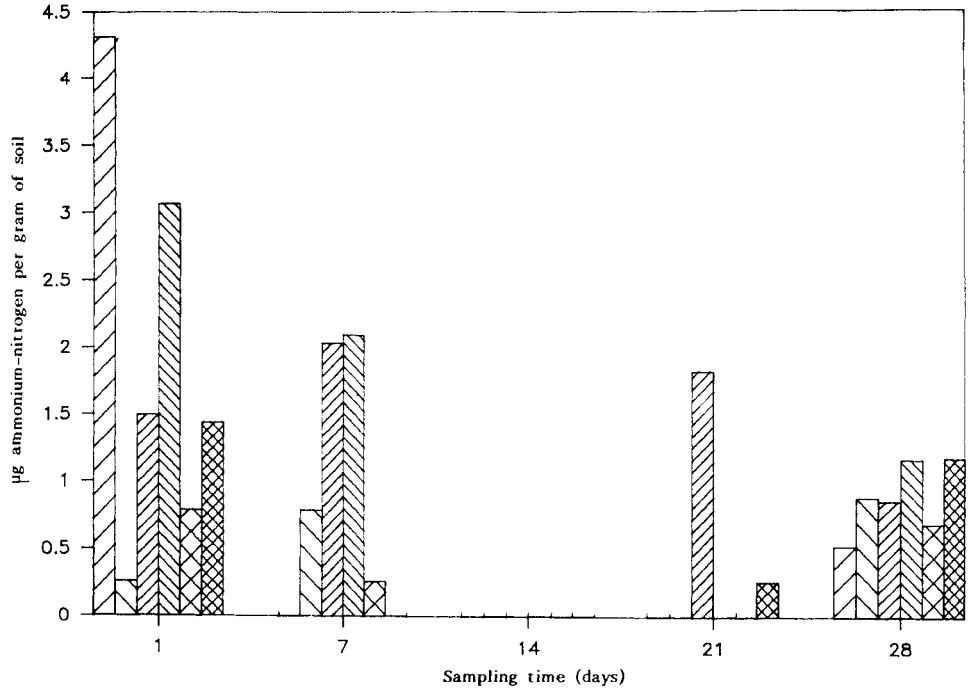

Figure 10 Effect of *p*-tolyldiphenyltin acetate on ammonification in soil.

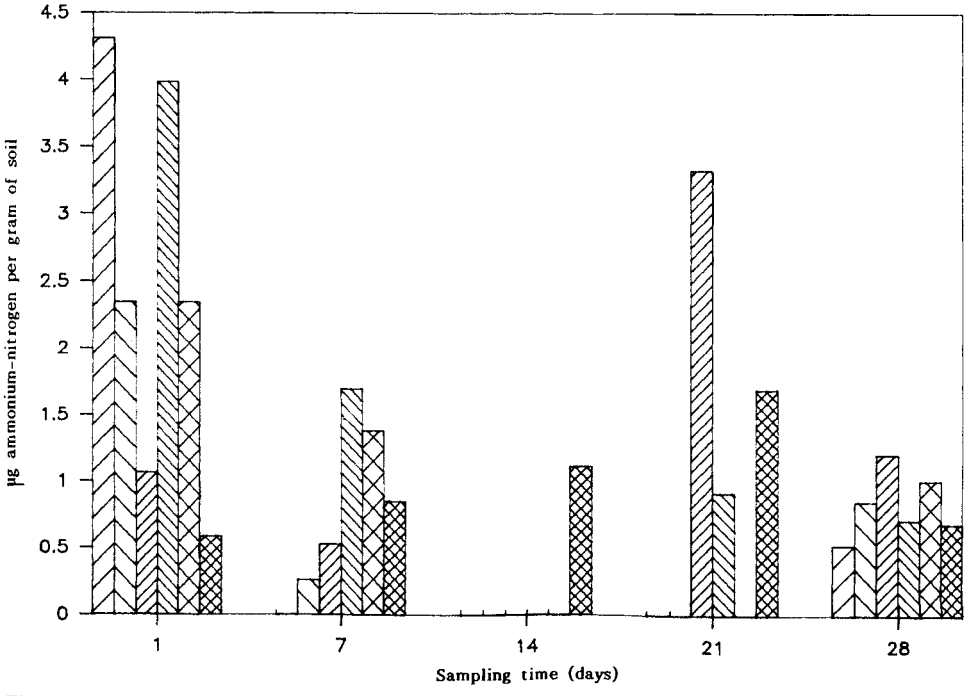

Figure 11 Effect of triphenyltin chloride·triphenylphosphine oxide on ammonification in soil.

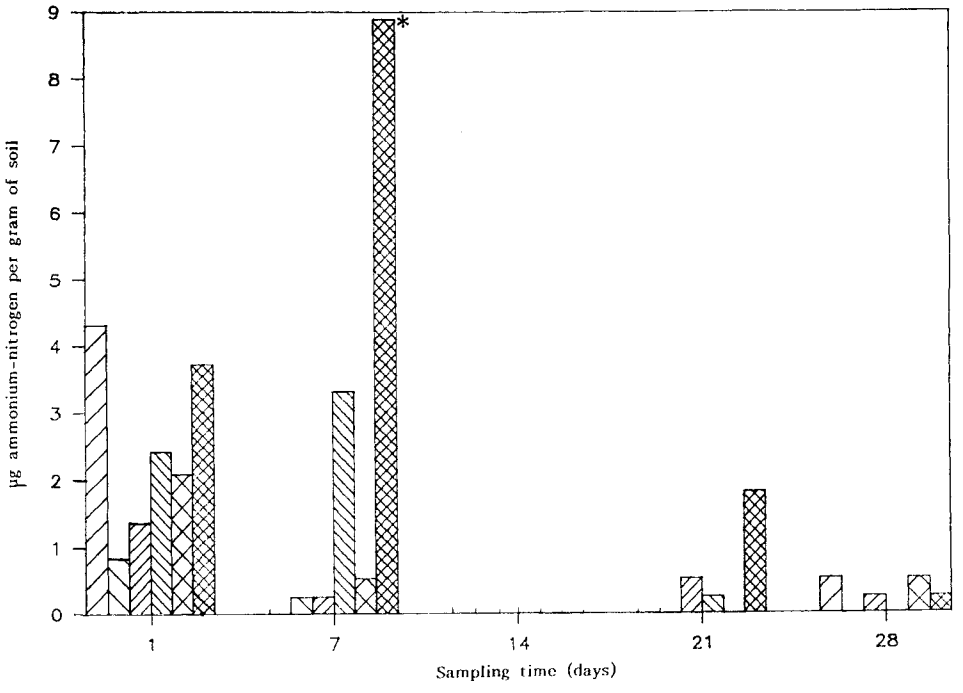

Figure 12 Effect of diphenylbutyltin bromide on ammonification in soil.

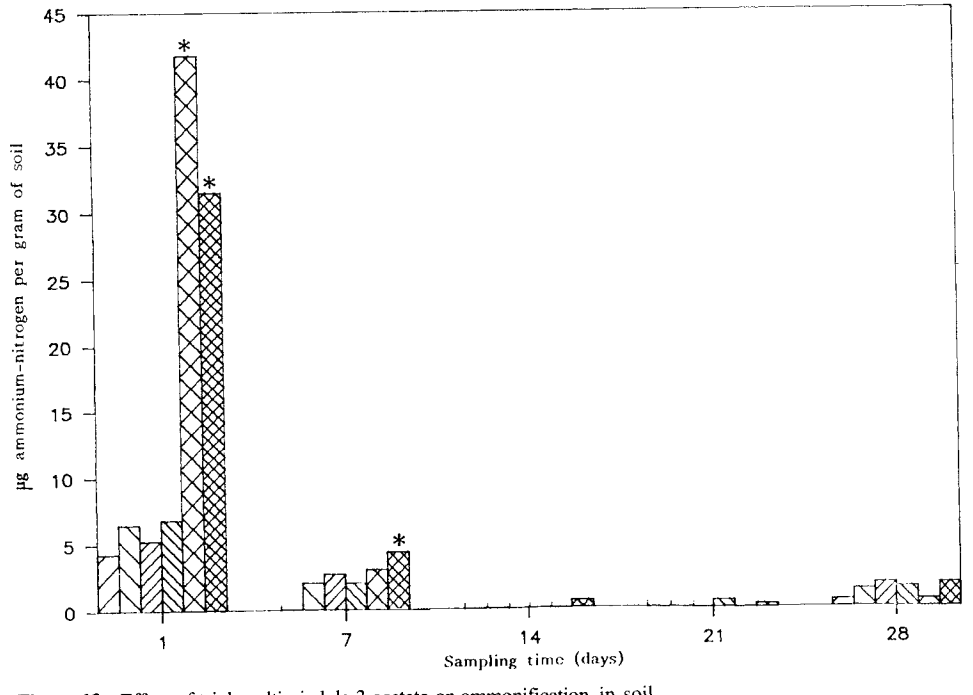

Figure 13 Effect of triphenyltin indole-3-acetate on ammonification in soil.

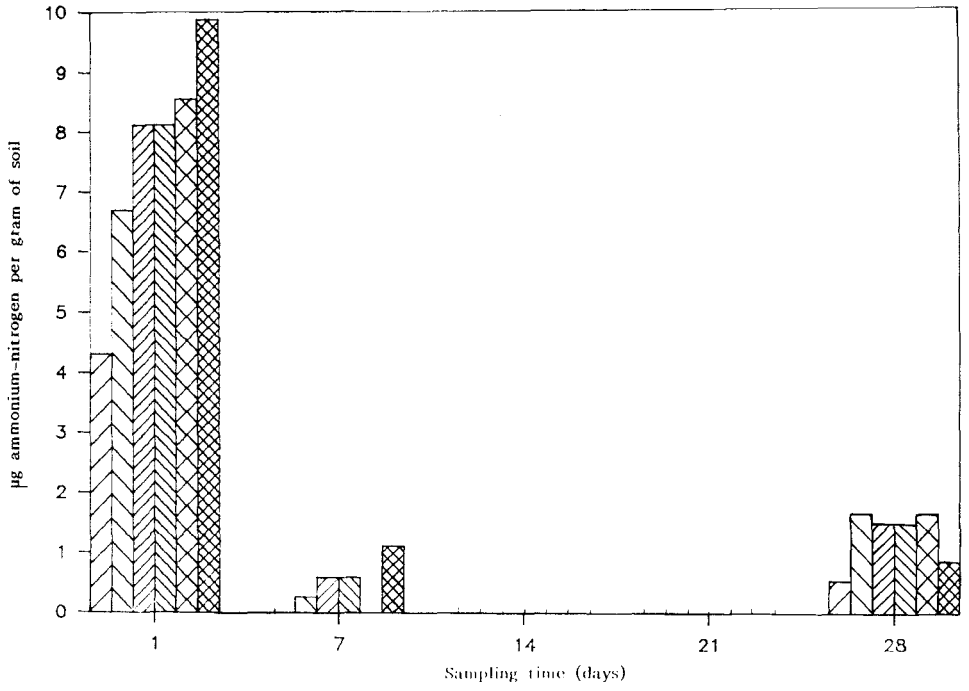

Figure 14 Effect of 2-ethylamino-4-triphenylstannoxy-5-n-methyl-6-butylpyrimidine on ammonification in soil.

Ammonium-nitrogen production at 100–250 $\mu g\ g^{-1}$ of Thiram and the triorganotin(IV) compounds follows the activity order:

> Thiram > diphenylbutyltin bromide > triphenyltin chloride·triphenylphosphine oxide > triphenyltin acetate > *p*-tolyldiphenyltin acetate > triphenyltin indole-3-acetate > 2-ethylamino-4-triphenylstannoxy-5-n-butyl-6-methylpyrimidine.

When the results obtained for nitrification and ammonification were compared for all treatments, it was generally noted that when there was an increase in nitrification, a decrease in ammonification ensued, and *vice versa* with respect to concentration and sampling time. For example, in soil treated with triphenyltin acetate at 250 $\mu g\ g^{-1}$, nitrate-nitrogen was not detected on day 7 but 3.34 μg ammonium-nitrogen g^{-1} of soil was detected on the same day. Furthermore, in soil treated with *p*-tolyldiphenyltin acetate, ammonium-nitrogen was not detected at all on day 14 but 1.97–23.22 μg nitrate-nitrogen g^{-1} of soil was detected on the same day.

DISCUSSION

Low concentrations of 10–50 $\mu g\ g^{-1}$ of Thiram and the triorganotin(IV) compounds except triphenyltin indole-3-acetate and 2-ethylamino-4-triphenylstannoxy-5-n-butyl-6-methylpyrimidine enhanced nitrification whereas high concentrations of 100 and 250 $\mu g\ g^{-1}$ inhibited nitrification. Similarly, Wainwright and Pugh[4] and Jacques *et al.*[34] noted enhanced nitrification preceded by an initial period of inhibition of nitrification associated with low concentrations of Milstem, Milcol, Captan and the dithiocarbamates. The stimulation of nitrification was most marked after treatment with triphenyltin acetate at 10 $\mu g\ g^{-1}$ of soil. In contrast, Barnes *et al.*[10] showed that triphenyltin acetate at 10 ppm ($\mu g\ g^{-1}$) did not cause any changes in nitrification in soil. Nitrate-nitrogen production was, however, measured at 20 h intervals for five days only in the study carried out by Barnes *et al.*[10]

The reverse was true for ammonification, where low concentrations of Thiram and the triorganotin(IV) compounds retarded or inhibited ammonification and high

concentrations enhanced ammonification. Ammonification was also enhanced during the initial period of incubation, i.e. day 1, except with triphenyltin acetate where high ammonium-nitrogen production was observed on day 7 instead. This implies that at high concentrations of Thiram and the triorganotin(IV) compounds, and during the initial period of incubation, NH_4^+ was not oxidized to NO_3^- as the nitrifying micro-organisms were then inhibited. Verdasan,[4,23] Nitrapyrin[35] and Terrazole[36,37] inhibited nitrification at $1-5\ \mu g\ g^{-1}$ while simultaneously enhancing ammonification. In this study, inhibition of nitrification and maximum ammonification were obtained at $250\ \mu g\ g^{-1}$ of Thiram, triphenyltin acetate, *p*-tolyldiphenyltin acetate, triphenyltin chloride·triphenylphosphine oxide and diphenylbutyltin bromide and at $>250\ \mu g\ g^{-1}$ of triphenyltin indole-3-acetate and 2-ethylamino-4-triphenylstannoxy-5-n-butyl-6-methylpyrimidine. Maximum nitrification and minimum ammonification occurred at $10\ \mu g\ g^{-1}$ of Thiram, triphenyltin acetate, *p*-tolyldiphenyltin acetate, triphenyltin chloride·triphenylphosphine oxide and diphenylbutyltin bromide. Triphenyltin indole-3-acetate and 2-ethylamino-4-triphenylstannoxy-5-n-butyl-6-methylpyrimidine did not exhibit any distinct patterns on nitrification and ammonification. Nitrification was always lower than for the control for these two triorganotin(IV) compounds, but ammonification was higher or similar to the control.

Nitrogen is one of the principal constituents of plants and it accounts for at least one half of the total number of ions absorbed by the plants.[38] Crop productivity depends mainly on the availability of this major element in soil. One of the major avenues of losses of nitrogenous fertilizers is through the process of nitrification where ammonia is oxidized to nitrate. The nitrification rate is also an important factor in nitrogen-cycling in soils where leaching is frequent.[39–42] Hence, the inhibition and the subsequent gradual recovery of nitrification with the triorganotin(IV) compounds at $250\ \mu g\ g^{-1}$ can be beneficial from the agronomic point of view, as the inhibition of nitrification helps to augment the nitrogen-use efficiency of added nitrogenous fertilizers by reducing the losses through nitrification. Furthermore, the inhibition of nitrification accompanied by the concurrent increase in ammonification by the triorganotin(IV) compounds at $250\ \mu g\ g^{-1}$ can be beneficial as nitrogen in the NH_4^+ form is held by cation exchange and is not easily leached from soil.

Nitrification is essentially a biological phenomenon mediated by specific chemo-autotrophic groups of bacteria such as *Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrosolobus*, and *Nitrobacter*.[43,44] The nitrifiers are believed[45] to be susceptible to partial sterilants. The ammonifiers, however, comprise a wide spectrum of organisms including bacteria, fungi and to a lesser extent the actinomycetes. As the various components of the microbial population of soil are differentially susceptible to the action of fungicides, the ammonification rate is not impaired even at high concentrations of the fungicides. The observed increase in ammonification in soils treated with the triorganotin(IV) compounds at $250\ \mu g\ g^{-1}$ could be due to the increase in heterotrophic bacteria after 14–21 days of incubation.[28] The increase in ammonification at $250\ \mu g\ g^{-1}$ of the triorganotin(IV) compounds could also be due to the increased dead microbial biomass made available resulting in rapid mineralization to NH_4^+. An initial decrease followed by a dramatic increase in the heterotrophic bacteria after the application of fungicides was also shown following treatment with Captan and Thiram.[9,46]

The increased nitrate production at $10-50\ \mu g\ g^{-1}$ of the triorganotin(IV) compounds after an initial period of inhibition may have resulted from the killing off of a portion of the soil microflora, especially the fungi. The unaffected ammonifying bacteria at the above low concentrations were thus able to utilize the newly available substrates to yield ammonium-nitrogen which acts as a 'primer' leading to increased release of ammonium-nitrogen from the organic matter. Oxidation of this substrate by the ammonium oxidizers then takes place and it is in turn rapidly oxidized to nitrate-nitrogen by the nitrite-nitrogen oxidizers. The enhanced nitrification after the application of triorganotin(IV) compounds at $10-50\ \mu g\ g^{-1}$ could also be due to a selective increase in *Aspergillus* and *Penicillium* which are active heterotrophic nitrifiers in pure culture.[47] Such an increase in these species was observed when the triorganotin(IV) compounds were added to soil.[28]

Among the triorganotin(IV) compounds, triphenyltin acetate, triphenyltin chloride·triphenylphosphine oxide, diphenylbutyltin bromide and *p*-tolyldiphenyltin acetate produced more favourable effects on nitrification and ammonification in soil than triphenyltin indole-3-acetate and 2-ethylamino-4-triphenylstannoxy-5-n-butyl-6-methylpyrimidine. These compounds enhanced ammonification while moderately inhibiting

nitrification at concentrations of 100–250 $\mu g\ g^{-1}$. From the results obtained in this study it appears that triphenyltin acetate, triphenyltin chloride·triphenylphosphine oxide, diphenylbutyltin bromide and *p*-tolyldiphenyltin acetate could be used effectively to control plant pathogenic fungi in soil at 100–250 $\mu g\ g^{-1}$ or 0.1–0.25 kg ha^{-1} concentrations without adversely affecting mineralization of nitrogen in soil. It is even probable that increased crop yields through controlled mineralization of nitrogen in soil could be achieved with these triorganotin(IV) compounds. Field studies on the triorganotin(IV) compounds are, however, required to augment these laboratory observations.

CONCLUSIONS

Concentrations of up to 50 $\mu g\ g^{-1}$ of triorganotin(IV) compounds enhance nitrate-nitrogen production in soil whereas concentrations of 100–250 $\mu g\ g^{-1}$ inhibit nitrification. The deleterious effects of the triorganotin(IV) compounds are less persistent compared with that of Thiram and recovery of nitrification is evident seven days after application of the triorganotin(IV) compounds. Low concentrations of triorganotin(IV) compounds inhibit ammonification whereas at higher concentrations the process is enhanced. Whilst complete inhibition of ammonification is achieved 21–24 days after application of Thiram, with the triorganotin(IV) compounds ammonification generally persists at all concentrations 48 days after application.

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