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EFFECTS OF PESTICIDES ON SOIL MICROBIOLOGICAL PROCESSES

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SUMMARY

The thesis can be divided into two parts. The first part is devoted to a comparison of the two methods, the steam distillation and Technicon automated methods, used for the determination of inorganic nitrogen.

To test the precision and accuracy of the two methods, a 5 ppm standard of both ammonium and nitrate nitrogen was used for the steam distillation method and a 1 ppm standard of both ammonium and nitrate nitrogen was used for the Technicon method.

By the steam distillation method the % recovery for the NH_4-N standard was 95.2% and for NO_3-N 94%. The major factor affecting the recovery of nitrogen was the loss of ammonia at the joint between the spray trap and the distillation flask. The joint on the apparatus was modified and tests achieved the recoveries for NH_4-N of 99.6% and NO_3-N of 99.6%.

The inorganic-N was then determined by the Technicon AutoAnalyzer II. 1 ppm standards of both ammonium and nitrate-N prepared in water and 2M KCl were analysed. Standard deviations were 0.0275 and 0.0025 in water and KCl respectively for NH_4-N and 0.0056 and 0.0051 in water and KCl for NO_3-N. The standard deviation of NH_4-N in water was very high. One source of variability was identified in that particular flasks consistently gave low results.

A possible reason for the variability is due to the adsorption of NH_4^+ ion by negative sites on the glass walls of the volumetric flasks. NO_3^- ion would not be affected by such negative adsorption sites and in KCl, K^+ would be adsorbed in preference to NH_4^+ as K^+ would be present at a much higher concentration.

Both steam distillation and Technicon methods are found suitable in their own places. Though distillation procedures are time consuming they are simple, accurate and less expensive and not affected by the various organic and inorganic substances present in soil extracts.

Using the Technicon AutoAnalyzer II a concentration as low as 0.001 $\mu g/ml$ can be detected and large numbers of samples can be analysed quickly with a high degree of reproducibility. But these automated methods are very sophisticated and it is very difficult to run such complex systems which require a skilled operator and well furnished laboratories. So for developing countries steam distillation methods are more preferable.

The second part of the thesis is devoted to testing the effects of three pesticides, captafol, carbaryl and malathion on three selected microbial processes, the phosphatase, dehydrogenase activities and nitrification in two soils of different properties. The two soils used were Darvel Series, a sandy clay loam of pH 6.4 and 8.82% organic matter content and Dreghorn Series, a sandy soil

of pH 6.8 and 5.64% of organic matter content.

Phosphatase activity involved the colorimetric estimation of p-nitrophenol released from p-nitrophenyl phosphate after incubation at 37°C for one hour. Dehydrogenase activity involved the reduction of 2,3,5-triphenyl tetrazolium chloride to the red coloured triphenyl formazan after incubation at 37°C for 24 hours.

A significant decrease was observed in the activity of both enzymes with captafol and malathion at both levels of application (10 and 100 mg/kg) for both soils. Carbaryl at both levels of application significantly reduced both enzymic activities in Darvel soil. The low level of carbaryl did not affect either of the enzymic activities in Dreghorn soil while at high level this compound affected the dehydrogenase activity but not the phosphatase activity of the same soil.

Nitrification involved the incubation of soils treated with ammonium sulphate at 25°C, subsampling at intervals, extraction of inorganic-N using potassium sulphate followed by analysis by Technicon AutoAnalyzer II.

All three pesticides at 100 mg/kg significantly reduced the nitrification rate of both Darvel and Dreghorn soils. At 10 mg/kg all pesticides used but not malathion also significantly reduced the nitrification rate of Darvel soil. But the low level of all three

pesticides did not significantly affect the nitrification rate of Dreghorn soil.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 IMPORTANCE OF PESTICIDE USE

Pests and diseases are those organisms which compete with man for his agricultural land, his growing crops, and his harvested produce. The control of these pests and diseases is thus a problem as old as agriculture. History contains many references to seasons of high pest incidence, from the biblical plagues of Egypt to the failure of the Irish potato crop in the middle of the last century (Newman, 1978).

To control the pest and diseases Agriculture annually treats millions of acres of soil with large amounts of pesticidal chemicals, together with various carriers, diluents and solvents. The potency of many of these chemicals is extremely great. The amount of soil applied pesticide which reaches its target e.g. plant roots or insects, is generally less than 10%, and often less than 1% of that applied. However, much of the remainder may reach non target organisms, for example microorganisms which subsequently absorb and degrade the residues to naturally occurring chemicals (Riley, 1976).

The use of agrochemicals has been increasing continuously since World War II. The use of herbicides has been expanding more rapidly than other pesticides.

The total world sales of pesticides in 1978 was $8.3 billion (Edwards, 1989). Somerville (1988) also reported that pests are destroying about 35% of all potential food crops before harvest. These losses are primarily due to insects, plant pathogens, and weeds. After the crops are harvested an additional 10-20% is destroyed by insects, microorganisms, rodents, and birds. The worldwide sales of pesticides in 1986 were some three million tonnes.

Even in developing countries, where labour for weed control is relatively cheap, large quantities of herbicides are used, including a wide range of chemical compounds of greatly differing structures, spectrum of toxicity to living organisms, and persistence in soil. Hence, concern about the effects of these chemicals on soil ecosystems, which depend greatly on living organisms for maintaining long-term fertility, is fully justified (Edwards, 1989).

No one can doubt that the pesticide use has made enormous contribution to agriculture and public health. Without pesticides the world food supplies would be inadequate for the growing human population. The growth rate of world population is about 73 million people per year. Our globe will have a population of 6407 million people in the year 2000 (Knusli, 1979), therefore, the need for more food and the control of human disease factors will require even more use of pesticides which until now has played a more significant role than other

tools in increasing food production and in saving man's life.

However, the widespread use of these chemicals affects soil microorganisms and their activities significantly. Any of the various chemicals used as pesticides are capable of inhibiting or destroying at least certain soil microorganisms under certain conditions. We do not know enough to identify and quantify those effects of pesticides which may harm soil microbial function and in particular, soil fertility (Edwards, 1989). The magnitude and distribution of side effects of pesticides are influenced greatly by the persistence of the pesticide. Many scientists have feared that the wide use of toxic chemicals may have serious and long-term effects on microorganisms.

A number of investigators have spent considerable time in the past few years, researching the effects of pesticides on the environment. However, very little work has been carried out in the field concerning pesticide effects on the soil microorganisms although interest is now growing in this area.

A draft has been prepared by the Federal Biological Research Centre for Agriculture and Forestry, Federal Republic of Germany, outlining guidelines for the official testing of plant protection products in 1987. This report describes the tests that can be used to determine whether or not a pesticide is having an effect

on soil microorganisms. The effect of a pesticide on soil microorganisms is dependent on several factors, for example, the toxicity, mode of action, persistence, application rate and time of application. Pesticides can have many different effects, inhibiting certain enzymic routes permanently or temporarily, decreasing biochemical reactions or increasing their rates.

Microorganisms are important to soil fertility because they transform potential nutrients into active or available forms, and CO_2 production is a significant index of their activity. The pesticides can kill and have direct effects upon decomposer microorganisms, rhizospheric microorganisms, root pathogen, and disease antagonists. The populations of soil microorganisms are extremely large and they differ greatly in function, different groups being involved in such functions as organic matter breakdown, soil respiration and nutrient cycling. So the most frequent consequences of effects of pesticides on soil microorganisms will be those relating to soil fertility. The effects of pesticides on soil microorganisms and some of these key dynamic soil processes are mentioned below.

1.2 EFFECTS OF PESTICIDES ON SOIL MICROORGANISMS

Soils contain five major groups of microorganisms: the bacteria, fungi, algae, actinomycetes and protozoa. The weight of these organisms is referred to as biomass.

The biomass is usually expressed as liveweight in kg ha^{-1} to a certain depth. The microbial populations in soil are extremely large. Bacteria are the most numerous soil microorganisms ranging from 10^6 to 10^9 bacteria per gram of soil. The numbers of actinomycetes range from 10^5 to 10^8 per gram of soil, and fungi range from 10^4 to 10^7 per gram of soil. This represents a biomass of 300 to 3000 kg/ha of bacteria, 700 kg/ha of actinomycetes, and 500 to 5000 kg/ha of fungi (Alexander, 1977, and Edwards, 1989).

It is possible to assess the effects of pesticide on microbial biomass, and several methods have been been suggested for estimating microbial biomass. These include measurements of ATP and muramic acid. The biomass also may be calculated from the biovolume by multiplying the number of organisms in a soil sample by the volume of an organism of medium volume (Edwards, 1989).

Another method was proposed by Jenkinson and Powlson (1976), who deduced the biomass of the microorganisms from the measurement of the flush of CO_2 evolved after fumigation of the soil with $CHCl_3$. They noted that the CO_2 evolved came from carbon mineralization of the bodies of the microorganisms killed by the treatment; 50% of this carbon was mineralized during the 10 days after the treatment by the surviving or reinoculated microorganisms recolonizing the medium.

The literature contains little evidence of any major population changes in genera or species of microorganisms after treatment with pesticide. The significance of the effects of herbicides on individual species or groups of microorganisms is virtually impossible to assess. A change in the species composition of soil microorganisms may occur after application of herbicide, but complete elimination of any single species is extremely unlikely. In all attempts to interpret experiments which tested the effects of pesticide in soil microorganisms, the "Sterilization phenomenon" must be considered because subsequent stimulation of microbial populations and activities will be much greater, the stronger the preceding biocidal action (Edwards, 1989). Moreover, in laboratory experiments, physical disturbance of soil can stimulate microbial activity markedly for a period up to 3 weeks (Jenkinson and Powlson, 1976). So any effect of a pesticide on microbial activity must be measured by taking this stimulation into account. Another complication in interpretation of results is that many microorganisms can use pesticides as substrate, thereby increasing microbial activity after treatment, so that adverse effects on microbial activity relates the balance between such increases and any adverse effects from the chemical (Edwards, 1989).

Cole (1976) indicated that long term atrazine use had little effect upon soil microbes. The effect of

Garlon-3A at rates of 2000 and 4000 ml/ha on microbial activity was found to be slightly toxic (Sapoundjieva, 1987). Tu (1989) found some transitional effects of the insecticides on microbial numbers and activities, but these effects were neither drastic nor prolonged enough to be considered deleterious to soil microorganisms and their activities important to soil fertility.

Bacteria, fungi, and algae differ in their responses to pesticides; responses are often related to the biochemical mechanisms of action of the pesticide and pesticide concentrations in the soil. Total microbial populations in the soils are often unaffected or only slightly affected by the pesticide applications, but populations and activities of individual species or groups of microorganisms may be greatly affected. Pesticide concentrations greater than those resulting from field application can cause interruption of microbial activities and shifts in populations. Most pesticides used at field rate do not appear to cause significant lasting effects on the microbial activities most related to soil fertility. In general, the changes in microbial populations due to pesticide applications are not more severe than changes caused by natural environmental stresses (Moorman, 1989).

1.2.1 EFFECTS OF PESTICIDES ON BACTERIA

Reports on the effects of pesticides on bacteria are extremely conflicting. The great majority of herbicides have been reported to have no effect on bacteria when applied at normal dosages (Grossbard, 1976). These include cycluron, chlorbufam, endothal, dalapon, trichloracetate, chlorazine, cycloate, lanacil, propham, pyrazon, DNOC (2-methyl-4,6-dinitrophenol), TCA (trichloro acetic acid), DCPA (dichlorophenoxyacetic acid) and some substituted ureas (Edwards, 1989). Triazines, atrazine and simazine also seem to not affect bacteria (Houseworth and Tweedy, 1973; Voets et al., 1974).

A few workers have reported some inhibition of bacteria by herbicides at field dosages. These include dichlobenil, dalapon, aminotriazole, and diallate (Grossbard, 1976 and Chandra, 1964).

Some herbicides can stimulate the growth of bacteria because they provide a food substrate for the bacteria or increase the availability of decaying organic matter. These include paraquat (Tu and Bollen, 1968) and sethoxydim (Roslyeky, 1986). Linuron, cycluron and chlorbufam can also stimulate activity at recommended doses. Some herbicides belonging to the phenoxyacetic group, have no effect on bacterial populations when applied at normal field rates, e.g. 2,4-D and 2,4,5-T, but these can stimulate activity at doses 100 and 1000

fold higher than normal. In some cases triazines have caused a stimulating effect on bacteria when applied with methoprotryne or a transient depressive effect, followed by stimulation by atrazine and simazine. Some herbicides cause depressive effects, e.g. dinoseb which is more toxic than bentazone and reduces bacterial populations only at high rates. In some cases, the initial depressive effects are also followed by an increase in bacterial numbers beyond the normal level. Other examples of such increases in bacterial activity by herbicides include chlorazine, cycluron, and chlorfuban (Edwards, 1989).

Van Faassen (1974) reported that repeated application of fungicide benomyl or the use of a high dose of benomyl can affect the total numbers of bacteria and may cause a shift in the bacterial flora of the soil. Anderson et al. (1981) reported that three fungicides captan, thiram and verdasan at the rate of 5 $\mu g\ g^{-1}$ caused significant decreases (40%) in the biomass. At 50 $\mu g\ g^{-1}$ the fungicides caused long-term decreases in the biomass. Verdasan had the greatest effect on soil microbial numbers. But Wainwright and Pugh (1974) reported that the field application of six fungicides at twice the normal rate resulted in increases in bacterial numbers after 20 days.

Past reviews of the effects of insecticides on soil microbial populations and activities have stressed the

fact that insecticides do not in general have much effect, except at concentrations greatly exceeding normal recommended field rates. While this is undoubtedly still true, there is increasing evidence to suggest that even at normal field rates insecticides may have some impact on soil microorganisms (Anderson, 1978).

Aldrin, chlordane, dieldrin and heptachlor at very high rates (10000 ppm) have a selective effect on bacterial growth and inhibited a wide range of gram-positive organisms without affecting gram-negative types (Anderson, 1978). Heptachlor (25 ppm) killed 63% of the soil bacterial isolates while at 100 ppm, 89% of the isolates were prevented from developing (Shamiyeh and Johnson, 1973). Diazinon at 3000 ppm inhibited the growth of bacterial isolates although one degradation product had no inhibitory effect while another slightly increased growth (Robson and Gunner, 1970).

Gawaad et al., (1972b), using sandy loam, calcareous clay and clay soils, tested the effects of chlordecone, endrin and lindane at the rate of 1.0 kg ha^{-1} and pirimiphos-ethyl and fonofos at the rate of 0.5 kg ha^{-1}. During the first week after the pesticide applications, numbers of bacteria gradually decreased, but rapidly increased in the second week and then gradually reverted to normal.

In reviewing earlier works Anderson (1978) reported that lindane, at high levels (6000 kg ha^{-1}) or toxaphene

at 600-6000 kg ha^{-1} caused a slight stimulation of bacteria for a short period, while anaerobic and spore-forming bacterial populations decreased in drier conditions. In greenhouse incubated loam soil, treated with parathion-methyl (288 kg a.i. ha^{-1}), the total bacterial population numbers increased during the first five days after treatment, decreased over the next 10 days and then increased greatly above the control level for the next 20-25 days. Using a wider concentration range of parathion-methyl (0.15 to 150 kg a.i. ha^{-1}) in the same soil-type, incubated under greenhouse conditions, the lowest rates gave brief increases in bacterial numbers and the highest rate resulted in greater increases. The loam soil, under field conditions, showed an increased population with the high rate that lasted for 26 weeks but the increase was delayed in dry years. Anaerobic bacteria were initially suppressed with rates of 0.15-300 kg a.i. ha^{-1} but then increased. The same insecticide at the rate of 1.5 kg a.i. ha^{-1} added to fallow loam and loam under vegetative cover revealed slight decreases and increases in bacterial numbers for a period of 20 weeks in the former but a significantly decreased population for the first 5 weeks followed by a recovery over the next 15 weeks in the latter. Dieldrin at 200 kg ha^{-1} had no effect on bacterial population in soil and a similar effect was obtained with aldrin, diazinon and thionazin at 10 kg

ha^{-1}.

Venkatramesh and Agnihothrudu (1988) reported that soil bacterial populations increased with increasing concentrations of captafol significantly in alluvial soil at 100 ppm. Ammonium sulphate also significantly increased bacteria in laterite soils.

1.2.2 EFFECTS OF PESTICIDES ON FUNGI

Fungi fall into two main classes. First, there are those that contribute to soil fertility by breaking down organic matter and those that facilitate nutrient uptake. Second, there are the plant pathogenic fungi which are responsible for plant diseases. Most herbicides, at normal dosages, have not been reported to have any effects on overall soil fungal populations. In particular, sodium chlorate (Karki et al., 1973) and atrazine and simazine (Voets et al., 1974).

A few herbicides have been reported to depress fungal activity (Grossbard, 1976). These include atrazine and cyanazine (Abdel-Fattah et al., 1983), simazine and atrazine, pyrazon, cycluron, propachlor, 2,4-D, paraquat, and ametryn (Zharasov et al., 1972; Camper et al., 1973). Basta in some cases inhibited fungal growth depending on culture conditions (Malkomes, 1988).

There have been some reports of slight stimulation of fungal activity by herbicides such as pyramin,

prometryn, dinoseb, 2,4-D, alachlor and fluchloralin (Edwards, 1989).

The effects of herbicides prometryn, diuron, fluometuron and MSMA (Monosodium methanearsonate) at the concentrations of 0, 10^{-6}, 10^{-5} and 10^{-4} M were used to measure their effects on colony diameter growth, gain in mycelial dry weight, sporulation and respiration of the two fungi. No concentration of any of the herbicides had any major effect on any of these parameters except sporulation (Davis et al., 1976). Fungi are not very susceptible to herbicides.

The fungicide MMDD (methylmercury dicyandiamide) was found to be active at only 1 ppm against fungal cultures, whereas thiram, maneb, and especially captan, were less toxic (Chinn, 1973). Helling et al. (1974) found that the fungus _Trichoderma_ _viride_ was sensitive to the greatest number of fungicides of the ten fungi used for bioassay.

Benomyl (4 and 20 kg ha^{-1}), captan (9 kg ha^{-1}), quintozene (5.6 and 11.2 kg ha^{-1}) and thiram (6.7 or 13.4 kg ha^{-1}) applied to field plots of a soil caused increases in fungal propagules 28 days after treatment (Wainwright and Pugh, 1974). Four fungicides, captan, dicloran, thiram and verdasan were applied at 28 days interval for 12 consecutive months. These fungicides reduced the number of fungus propagules in soil by 23, 11, 36, and 50% respectively compared with control.

Captan and dicloran treated soils rapidly recolonised within 7 days of the application of fungicides. The effects of thiram and verdasan were more persistent, the fungal numbers in soils treated with these fungicides did not recover sufficiently to reach control level throughout the sampling period (Kuthubutheen and Pugh, 1979). Dickinson (1973) indicates that a significant proportion of leaf microbes are susceptible to fungicide, especially those known to have a wide spectrum of activity against pathogens.

The insecticide aldrin inhibited 7 out of 17 fungi while lindane affected 15, parathirn 16, phorate 13 and carbaryl 15 out of 17 fungi (Cowley and Lichtenstein, 1970). Naumann (1970a) found that parathion-methyl at high rates (150 and 300 kg a.i. ha^{-1}) decreased the numbers of fungal propagules and altered the species spectrum. Other insecticides which have been reported to decrease fungal populations include lindane, endrin, chlordane (22kg a.i. ha^{-1}), pirimicarb and fonofos at the rate of 11 kg a.i. ha^{-1} (Gawaad et al., 1972b, 1973a). Carbaryl and DDT at field rates completely inhibited a number of saprophytic fungi in soil, but in some cases fungi which were susceptible to DDT were found to be stimulated by carbaryl (Varshney and Gaur, 1972).

1.2.3 EFFECTS OF PESTICIDES ON ACTINOMYCETES

Actinomycetes populations in soil do not seem to be affected much by herbicides. They are not reported to be affected by the phenoxy acids, DNOC (2-methyl-4,6-dinitrophenol), sodium chlorate, calcium cyanamide or substituted ureas (Edwards, 1989), paraquat (Camper, 1973) and simazine (Kulinska, 1967).

Some herbicides have been reported to depress actinomycetes activity which include dalapon and EPTC (ethyl dipropyl thiolcarbamate). A few herbicides were reported to stimulate the actinomycetes activity, e.g. prometryn, dinoseb, and 2,4-DB [4-(2,4-dichloro phenoxy)butyric acid] (Edwards, 1989).

A fungicide quintozene, at the rates up to 20 kg ha^{-1} in loam soils, produced no changes in numbers of actinomycetes but in soil amended with various energy sources, chitin in particular, quintozine strongly suppressed the numbers of actinomycetes (Farley and Lockwood, 1968, 1969). Captan decreased the numbers of actinomycetes (Mahmoud et al., 1972), but the soil samples treated with benomyl (100 ppm) did not influence the numbers of actinomycetes (van Faassen, 1974).

Insecticides at normal field application rates have little effect on actinomycetes population, but they are more sensitive in culture than in soil (Anderson, 1978). Naumann (1970a) found that parathion-methyl at 0.15, 1.5, 15, 150 or 300 kg ha^{-1} significantly increased the

numbers of actinomycetes in loam soil. Samples of sandy loam, calcareous clay and clay soils treated with lindane, endrin or chlordecone (20 kg a.i. ha^{-1}) and pirimicarb or fonofos (11 kg a.i. ha^{-1}) decreased actinomycete populations during the first week, followed by a rapid increase two weeks after treatment and finally return to pretreatment population numbers (Gawaad et al., 1972b, 1973a).

1.2.4 EFFECTS OF PESTICIDES ON SOIL ALGAE

The population of soil algae fluctuates greatly and they comprise between 4 and 27% of the total microbial biomass in soil (McCann and Cullimore, 1979). They probably contribute to both carbon and nitrogen cycles in soil, but there is little evidence that algae are important in soils or have any influence on soil fertility (Edwards, 1989).

The effects of metribuzin and two analogs on five species of algae were studied by Arvik et al. (1973) and with one exception, the growth and numbers of all of the tested algae were significantly reduced by increasing herbicide concentrations. Other herbicides which have been reported to depress algal populations include lenacil, pyrazone, propazine, atrazine, diuron, propanil, atrazine (Edwards, 1989) and chlorpropham (Maule and Wright, 1984).

The sensitivity of algae to herbicides depends on the structure of the herbicide, but Chalamydomonas and Chlorococcum tend to be more sensitive than Chlorella and Nostoc, based on studies on 2,4-D, trifluralin, MCPA (2-methyl-4-chlorophenoxyacetic acid), and TCA (trichloroacetic acid) (Cullimore and McCann, 1977).

Harriss et al. (1970) found that three organo-mercury fungicides, at less than 1 ppb, reduced growth and photosynthesis in the marine diatom Nitzschia delicatissima and also in a natural population of fresh water phytoplakton. Moore (1970) reported that maneb was found to be more inhibitory than zineb to the growth of Euglena gracilis while nabam was toxic at all levels tested. Chlorella sorokiniana was found sensitive to most of the 33 fungicides tested on soil TLC plates by Helling et al. (1974). Gupta and Saxena (1974) tested panacide, a formulation of dichlorophen, for its effects on cultures of green and blue green algal species. The blue green Nostoc sp. was found to be the most sensitive, even though it survived up to 10 ppm Panacide. Canton (1976) reported that MBC (methyl benzimidazol-2-yl carbamate) was more toxic than benomyl or thiophanate-methyl towards Chlorella pyrenoidosa, EC_{50} values of 0.34, 1.4 and 8.5 ppm respectively.

Moore (1970) found that malathion and parathion were relatively non inhibitory to the flagellate Euglena gracilis. Initial inhibition of N_2-fixation in

blue-green algae caused by 100 ppm malathion was followed by recovery and stimulation (Da Silva et al., 1975). Diazinon (1 ppm) had no effect on cell numbers or photosynthesis in Scenedesmus quadricuada (Standnyk et al., 1971). Dursban applied at normal rate (1.2 ppb) to a pond, exerted a persistent reduction in growth of most of the phytoplankton (Brown et al., 1976). At 2.4 ppb, however, dursban had been found to stimulate growth of blue-green algae in artificial ponds (Wright, 1978).

Christie (1969) found that at 100 ppm carbaryl reduced the population of algae. Marine algae were also susceptible to carbaryl, which was lethal to two species at 1 ppm and to all five species tested at 10 ppm (Ukeles, 1962).

1.3 EFFECT OF PESTICIDES ON ORGANIC MATTER BREAKDOWN

The breakdown of organic matter seems to be the best and most important process occurring in soils since it is the major source of nutrients, therefore it should be a key technique in assessing hazards due to the pesticides. There are surprisingly few papers reporting the effects of pesticides, such as herbicides, on organic matter degradation (Edwards, 1989).

The herbicides which have no effect on cellulose decomposition include atrazine, cycluron and chlorobufam, chlorthaldalapon, simazine, DCU (dichloral urea), chloropropham, dinoseb, monuron, 2,4-D, and simazine

(Edwards, 1989) and bromacil and methoprotryne (Wolf and Martin, 1975).

Some authors have reported that certain herbicides affect the decomposition of cellulose. These include atrazine and simazine (Voets et al., 1974), chlorbufam and DMPA [O-(2,4-dichlorophenyl)O-methyl N-isopropyl-phosphoroamidothiate], 2,4-dipropanil, and paraquat (Rankov and Velev, 1975).

Stimulation of cellulose breakdown by some herbicides has also been reported. These include linuron (Simon-Sylvestre and Fournier, 1979) and propachlor (Rankov and Velev, 1975). Thus, some workers have reported the inhibition of cellulose breakdown, while other workers reported no effects or stimulation of cellulose decomposition, all caused by the same herbicides. These conflicting results indicate that herbicides do not have any serious effects on organic matter breakdown (Edwards, 1989).

Fungicides may influence the degradation of cellulose and organic matter. Domsch (1970) reports that captan seriously delayed the formation of cellulases by soil organisms.

Benomyl (2 kg ha^{-1}) had no effect on the cellulolytic activity of organisms in sandy soils (van Faassen, 1974). Baroux and Sechet (1974), however, report that low rates of copper sulphate in a sand inhibited cellulolysis, but the effect could be overcome

by the addition of lime and colloidal humic acids.

In a study of the effects of 8 insecticides on decomposition of organic matter, only propoxur was found toxic to the microbial decomposition of organic matter in sewage (Lieberman and Alexander, 1981). Mahmoud et al. (1970) found that numbers of cellulose decomposers were stimulated by the insecticide lindane but decreased by dieldrin, although organic matter content of the soil was not affected by either insecticide. In a loam soil treated with parathion-methyl at 0.15 to 300 kg a.i. ha^{-1} the population of cellulose decomposers increased after a transient decrease, but under drier soil conditions the increase was delayed (Naumann, 1970a).

In a sandy silt-clay, glucose utilization was stimulated and then reduced by propoxur at 2.5 or 12.5 kg ha^{-1} and completely inhibited by 125 kg ha^{-1} (Gupta et al., 1975). In three surface soils the application of counter (0.1 to 100 kg ha^{-1}) had no effect on cellulose decomposition for six weeks (Laveglia and Dahm, 1974).

1.4 EFFECTS OF PESTICIDES ON SOIL RESPIRATION

Respiration measures are probably the most common methods of studying microbial activity in soil because respiration usually correlates well with other soil activities, such as transformation of carbon, phosphorus, and nitrogen. The evolution of carbon dioxide and uptake of oxygen are parameters related to many dynamic

processes in soil and particularly to the decomposition of organic matter. Measurement of total soil respiration can be made in a Warburg respirometer, after small soil samples are treated with a particular pesticide at appropriate doses (Edwards, 1989).

Many herbicides have been reported to inhibit soil respiration at unrealistically high doses. These include atrazine and barban (Bartha et al., 1967), bentazon, picloram, and trifluralin (Grossbard, 1976), chlorthiamid, fluometuron, and monuron (Simon-Sylvestre and Fournier, 1979); linuron, monuron, and metoxuron (Grossbard, 1974).

Herbicides that have been reported to inhibit soil respiration at field application doses include aminotriazole, dalapon and dichlorprop, ioxynil and mecoprop (Schreven et al., 1970), asulam and bentazone (Grossbard, 1976), diuron and EPTC (ethyl dipropyl thiolcarbamate) (Chandra, 1960).

Herbicides that reported to stimulate gaseous exchanges in soil include simazine and ioxynil (Smith and Weeraratna, 1974), chloroxuron, diuron, fluometuron, metabromuron, and monuron (Grossbard, 1974).

Fungicides dodine and captan at concentrations of 100-500 ppm or ethirimol at 400 and 500 ppm decreased the O_2-uptake by <u>Rhizobium</u> <u>trifolii</u> while lower concentrations of ethirimol (100-300 ppm) were stimulatory and dimethirimol, triforine, carboxin and

oxycarboxin had little effect (Fisher, 1976). In liquid media a 50% decrease in respiration rates of Fusarium oxysporum f. sp. melonis and Saccharomyces cerevisiae was caused by benomyl (3.5×10^{-6}M) and benomyl, thiabendazole, carbendazim, thiophanate and thiophanate-methyl at 250×10^{-6} M all inhibited mitochondrial respiration (Decallone et al., 1975). The benomyl does not have much inhibitory impact on soil respiration (Peeples, 1974 and van Faassen, 1974).

A fine sandy loam soil treated with lanstan at 1.0 and 10 kg ha^{-1} exhibited a depressed respiratory activity but quintozene and TCNB (1,2,4,5-tetrachloro-3-nitrobenzene) only affected O_2-uptake at concentrations of 100 kg ha^{-1} (Caseley and Broadbent, 1968). In soils with or without glucose, cellulose or chitin, captan inhibited respiration and the more resistant the substrate, the longer was the period of inhibited respiration (Domsch, 1970).

Lindane, aldrin, and dieldrin at the rate of 0.1-100 kg ha^{-1} in an alluvial soil, had no effect on respiration at lower rates, but higher rates decreased CO_2-evolution (Bardiya and Gaur, 1968). Samples of sandy loam calcareous clay and clay soil treated with lindae (22 kg a.i. ha^{-1}), thionazin and trichloronate at the rate of 1.0 and 10 kg ha^{-1} and fonofos at the rate of 11 kg a.i. ha^{-1}, initially decreased CO_2-evolution, then increased, and returned to normal (Gowaad et al., 1973a). But Tu

(1975) reported that lindane in a loamy sand at the rate of 1.0-100 kg a.i. ha^{-1} increased O_2-uptake.

Aldicarb in a sandy loam at the rate of 0.5 and 50 kg ha^{-1} had little effect on CO_2-evolution (Kuseske et al., 1974). In a sandy silt-clay, propoxur, at the rate of 2.5, 12.5, and 125 kg ha^{-1} decreased CO_2-evolution by two lowest rates, but recovered. High rate decreased CO_2-evolution after initial increase (Gupta et al., 1975). Diazinon and chlorpyrifos at the rate of 1.0 and 10 kg ha^{-1}, increased respiration in a sandy loam soil (Tu, 1970). 3 soils treated with counter at the rates of 0.1-10 kg ha^{-1} had no effect on CO_2-evolution (Laveglia and Dahm, 1974).

1.5 EFFECTS OF PESTICIDES ON AMMONIFICATION

Ammonification of organic material, resulting in the release of NH_4^+ ions, is a key process in soil supplying readily available nitrogen to plants and microorganisms. Without ammonification nitrification would be severely limited and NO_3^- ions, the preferred form of nitrogen for some plants and microorganisms, would become of limited availability. The fact that, in many cases, ammonification is stimulated by herbicide applications is not surprising since the death of plants and possibly much of their attendant rhizosphere and phyllosphere populations, will result in an increase in proteins available for transformation. Only the highest

concentrations of herbicides inhibited ammonification. The three largest groups of herbicides, the phenoxy-acids, triazines, and urea-type compounds are mainly positive in their effect on ammonifying microorganisms (Anderson, 1978).

Edwards (1989) reviewed that since few herbicides seem to affect the amount of mineral nitrogen, the formation of ammonium nitrogen is also unlikely to be affected because most reports in literature indicate that the ammonifying bacteria are relatively tolerant to herbicides, probably because of the great heterogenecity of the bacterial species found within this group. However, reports indicate a diversity in the effects of the pesticides on these organisms. In experiments set up specially to test ammonification, the process was affected very little (Simon-Sylvestre and Fournier, 1979).

None of the following herbicides had any effect on ammonification: simazine, atrazine, cyanazine, paraquat and trifluralin (Edwards, 1989). Other studies have indicated minor effects of herbicides or ammonification. These include metribuzin (Simon-Sylvestre and Fournier, 1979); and simazine and linuron (Grossbard, 1971).

A third group of herbicides has been reported to stimulate ammonification. These include simazine and atrazine (Voets et al., 1974), MCPA (2-methyl-4-chloro-phenoxyacetic acid) and 2,4,5-T (Trostensson, 1974).

Obviously, most herbicides do not significantly affect ammonification (Edwards, 1989).

The ammonification is generally favoured by fungicide treatment of soil. This may be due to decreased fungal growth and competition, or increased bacterial ammonification of the protein of killed fungi (Anderson, 1978). In an acid lateritic clay and an alluvial loam, maneb and anilazine at up to 24 kg ha^{-1} had no persistent effect on ammonification, but at 96 kg ha^{-1} inhibition occurred (Dubey and Rodriguez, 1970). Ammonification increased with increasing concentration of verdasan (0.1-5 kg ha^{-1}), thiram (0.5-25 kg ha^{-1}) and captan (0.5-25 kg ha^{-1}) applied to a soil (Wainwright and Pugh, 1973). In contrast, ammonification was inhibited with increasing concentration of TCMB [2-(thiocyano-methyl)benzothiazole] up to 30 kg ha^{-1} in a humus soil (Voets and Vandamme, 1970). Ammonification was also increased in soil treated with benomyl, captan, quintozene or thiram (Wainwright and Pugh, 1974), but was inhibited by low rates of copper sulphate in a sand inoculated with a soil suspension (Baroux and Sechet, 1974).

The effect of relatively large amount of common insecticides on microbial activity in soil was studied. Aldrine, chlordane, and DDT at rates of 50, 100 and 200 kg ha^{-1} did not have a significant effect on ammonification (Tewari et al., 1970). In clay soil

supplemented with 2% compost, lindane at 0.22 and 4.4 kg ha^{-1} or dieldrin at 2.5 and 50 kg ha^{-1} initially suppressed and subsequently increased ammonification. In loam soil, lindane at 500 kg ha^{-1} or campechlor at 50-500 kg ha^{-1} stimulated ammonification in moist soils but suppressed population numbers in drier soils (Anderson, 1978). The ammonification of peptone added to a sandy silt-clay was stimulated by propoxur at 2.5, 12.5 and 125 kg ha^{-1}, especially by the highest rate (Gupta et al., 1975). In a sandy clay loam or clay soil, endrin and chlordecone, each at 22 kg ha^{-1}, and fonofos or pirimiphos-ethyl at 11 kg ha^{-1} caused ammonification to fluctuate in the first four weeks after treatments were applied but activity returned to normal after seven to eight weeks (Gawaad et al., 1973b).

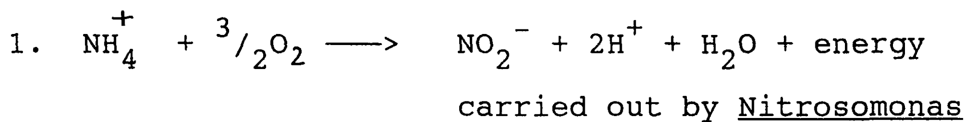
Diazinon, chlorpyrifos, thionazin and trichloronate each applied at 1.0 or 10 kg ha^{-1} to a sandy loam, increased ammonification in the soil (Tu, 1970). Parathion-methyl (0.15, 1.5, 15, 150 and 300 kg a.i. ha^{-1}), applied to a loam soil increased numbers of ammonifying microorganisms, but the three highest rates first transiently decreased them (Naumann, 1970a).

Counter, at rates of 0.1 to 10 kg ha^{-1} in three soil-types, had no effect on ammonification for six weeks (Laveglia and Dahm, 1974). Dichlorvos with urea or dieldrin with urea greatly influenced the ammonification (Satter and Morshed, 1989). The overall effect of

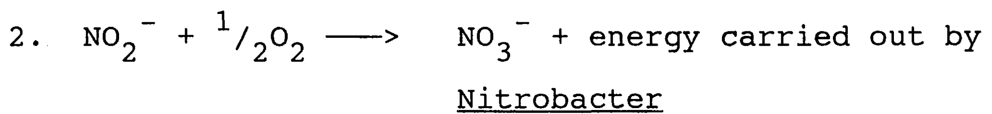
insecticides on ammonification is stimulatory rather than inhibitory.

1.6 EFFECTS OF PESTICIDES ON NITRIFICATION

Nitrification invloves the biological oxidation of inorganic nitrogen forms to nitrate (NO_3^-). The principal nitrifying organisms are chemoautotrophic bacteria: *Nitrosomonas* and *Nitrobacter*. These organisms are unique in deriving the energy they require for growth purely from the oxidation of ammonium (NH_4^+). The oxidation occurs in two steps:-

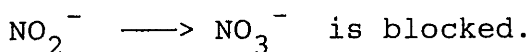


1. $NH_4^+ + {}^3/_2O_2 \longrightarrow NO_2^- + 2H^+ + H_2O$ + energy carried out by *Nitrosomonas*



2. $NO_2^- + {}^1/_2O_2 \longrightarrow NO_3^-$ + energy carried out by *Nitrobacter*

In the soil there are a few thousand ammonium oxidizers per gram of soil. However, *Nitrobacter* works faster so there should not be a build up of nitrite unless *Nitrobacter* is affected by the pesticide and the route



$NO_2^- \longrightarrow NO_3^-$ is blocked.

Some herbicides can affect nitrification. Consequently, the amount of nitrate formed in the treated soil is usually less than in the controls, and ammonium nitrogen accumulates. Those nitrifying bacteria which

oxidize ammonia in nitrates are usually very sensitive to stress. The mechanisms of nitrification are fragile and dependent on complex and sensitive enzymes (Edwards, 1989).

Most herbicides at recommended doses have not been reported to affect significantly either the nitrifying bacteria or nitrification. These include simazine , prometryn; neburon and fluometuron (Horowitz et al., 1974); dalapon and paraquat (Namdeo and Dube, 1973); and metamitron, phenmedipham, pyrazon, fenuron, 2,4-D and simazine (Edwards, 1989).

Prometryne has been reported to inhibit Nitrobacter in soil (Dubey, 1969).

Some studies report depressive results of herbicides on nitrification including MCPA (2-methyl-4-chloro-phenoxyacetic acid), 2,4,5-T, and linuron (Torstensson, 1974), atrazine (Voets et al., 1974) and prometryn, metabuzin, medipham, goltrix and sencor (Simon-Sylvestre and Fournier, 1979).

Other reports have demonstrated a stimulation of nitrifying bacteria by herbicides. Such effects have been observed after treatment with simazine and terbutryn (Smith and Weeraratna, 1974), prometryn (Simon-Sylvestre and Fournier, 1979), and bentazone (Grossbard, 1974).

Overall herbicides appear to have relatively little effect on nitrification. Simon-Sylvestre and Fournier (1979) suggested the following relationships which relate

the effects of the herbicide to the chemical configuration, and the persistence and effects of the herbicide in soil. In terms of inhibition:

1. The toxic effects decrease with time, indicating for example that either these products undergo a transformation (e.g., microbiological degradation), or that a resistant nitrifying population develops in the soil. Atrazine, EPTC (Ethyl dipropyl thiolcarbamate), malathion, and parathion are examples.

2. The inhibition of the nitrification remains constant throughout the test period. Such products are chemically and biologically stable. Chloranocryl, diphenamid, fenuron and monuron are examples.

3. The toxicity increasing with time, suggesting the formation in the soil of a product more harmful than the initial pesticide. Chlorpropham, diuron, and linuron are examples of this effect.

Ridomil in a gray forest soil had no effect on nitrification or nitrogen fixing bacteria at normal levels of application (Golovleva and Finkel'Shtein, 1988). Captan, thiram, and verdasan either stimulated or had no effect on nitrification in grass soil when applied at low concentrations of 0.5-10, 0.5-5, and 0.1-0.5 kg ha^{-1} respectively. At higher rates, captan (25 kg ha^{-1}),

thiram (10 and 25 kg ha^{-1}), and verdasan (1.0-5.0 kg ha^{-1}) inhibited the process (Wainwright and Pugh, 1973). In further experiments on an unspecified soil type, Wainwright and Pugh (1974) showed that captan (9 kg ha^{-1}), thiram (6.7 kg ha^{-1}), dicloran (2 kg ha^{-1}), and formalin (500 litres ha^{-1}) inhibited nitrification markedly whereas quintozene (5.6 kg ha^{-1}) had a slight effect. Mahmoud et al. (1972) also found that captan adversely affected soil nitrification.

Maneb, zineb, and tribasic copper, applied repeatedly to soil, resulted in decreased nitrification and nitrogen mineralization (Dubey, 1970). Anilazine and maneb at 1.5 to 96 kg ha^{-1} in acid lateritic clay and alluvial loam soils inhibited nitrification and _Nitrosomonas_ _spp_. but not _Nitrobacter_ _spp_., maneb being the more toxic (Dubey and Rodriguez, 1970).

In sandy soils receiving the equivalent of 1.0, 2.5, and 10 kg ha^{-1}, van Fassen (1974) found that benomyl had no effect for the first six weeks but then significantly increased nitrification in treated soil than in untreated soil. In mixed cultures of _Nitrosomonas_ _sp_. and _Nitrobacter_ _sp_., 20 ppm benomyl inhibited oxidation of NO_2^- to NO_3^- and that 200 ppm delayed the oxidation of NH_4^+ to NO_2^-, as well as the oxidation of NO_2^- to NO_3^- (van Faassen, 1974).

Lanstan at a concentration equivalent to 1.0 kg ha^{-1} and dicloran at 1.0 to 10 kg ha^{-1} inhibited nitrification

in a fine sandy loam but quintozene and tecnazene at up to 100 kg ha^{-1} had only a slight effect (Caseley and Broadbent, 1968). Fentin acetate at 1.0 kg ha^{-1} added to a loam soil initially decreased and then increased nitrification in one experiment but had no effect in a similar experiment (Barnes et al., 1973), while dexon in an unspecified soil inhibited nitrification at 35 kg ha^{-1} but not at lower rates of 1.1 to 1.4 kg ha^{-1} (Agnihotri, 1973).

Insecticides have inhibitory influence on nitrification. Carbofuran at 10 ppm inhibited nitrification and Nitrobacter was more sensitive to carbofunan than Nitrosomonas (Palaniappan and Balasubramanian, 1986). Lindane, malathion, and baygon were tested against Nitrosomonas europaea. Complete inhibition was obtained with all three compounds at 10 ppm, but delayed nitrification was not evident at lower concentrations (Garretson and San Clemente, 1968). Chlordane, TDE (Tetrachlorodiphenylethane), heptachlor or lindane at 10 ppm inhibited the growth of Nitrobacter agilis in aerated cultures but not by aldrin, while lindane, TDE (Tetrachlorodiphenylethane), heptachlor, and chlordane also partially inhibited NO_2^- oxidation (Winely and San Clemente, 1970). On the other hand, Mishra et al. (1972) found that either lindane nor phorate, at normal and 10 times normal rates, had any overall effect on nitrification although Nitrosomonas sp. was slightly

affected. Kuseske et al. (1974) found that at rates up to 500 ppm, propoxur and aldicarb were toxic to both Nitrosomonas sp. and Nitrobacter sp.

Shin-Chsiang Lin et al. (1972), using loam soil, tested the effects of eight insecticides on nitrification. Carbofuran and carbophenothion had no effect, trichlorfon inhibited at 5 and 50 kg ha^{-1} for a short period, fonofos, trichloronate and chlorpyrifos showed persistent inhibition at 50 kg ha^{-1}, and aldicarb and propoxur at 50 kg ha^{-1} showed marked inhibition of nitrification. Using small plots of a sandy clay loam and a clay soil, Gawaad et al. (1972b, 1973a) applied chlordecone or endrin at 22 kg ha^{-1} and fonofos or pirimiphos-ethyl at 11 kg ha^{-1} and found that nitrification decreased during the first two weeks, increased in the third and fourth weeks and then returned to normal after 7-8 weeks.

Lindane, aldrin, and dieldrin at 0.1, 1.0, 2.5, 10, and 100 kg ha^{-1} applied to a sandy loam soil, the lowest two rates of each had no effect on soil nitrification but all other higher rates inhibited the activity (Bardiya and Gaur, 1970). In clay soil supplemented with 2% compost lindane at 0.22 and 4.4 kg ha^{-1} or dieldrin at 2.5 and 50 kg ha^{-1} severely depressed the numbers of nitrifiers (Mahmoud et al., 1970). Lindane at recommended field rate had no overall effect on nitrification in soil (Mishra et al., 1972). According

to Ross (1974) DDT at 0.75, 10, and 50 kg ha^{-1}, applied to a silt loam, the lowest rate had no effect but other two high rates decreased the activity. Carbofuran at 0.75, 10 and 50 kg ha^{-1} in a silt loam, had slightly stimulatory effect on nitrification (Ross, 1974). Singh and Rana (1987) also reported that at the rate of 0.5, 1.0 and 2.0 kg ha^{-1}, carbofuran did not affect the NO_3-N level of the soil, but quinalphos stimulated the nitrification process at all three concentrations. Propoxur and aldicarb in a sandy loam at 0.5 kg a.i. ha^{-1} inhibited soil nitrification (Kuseske et al., 1974). Propoxur at 2.5, 12.5 and 125 kg ha^{-1} applied to sandy silt clay also inhibited the nitrification process (Gupta et al., 1975).

Diazinon, chlorpyrifos, thionazin and trichloronate each applied at 1.0 or 10 kg ha^{-1} to a sandy loam, either had no effect or slightly inhibited the nitrification process (Tu, 1970). Parathion-methyl at 0.15, 1.5, 15, 150 and 300 kg a.i. ha^{-1}, applied to a loam soil, decreased the numbers of nitrifying organisms by the lowest two rates, but top three rates initially decreased and then increased the numbers (Naumann, 1970a).

Counter, at rates of 0.1 to 10 kg ha^{-1} in three soil types had no effect on nitrification (Laveglia and Dahm, 1974). In most cases pesticides suppressed nitrification in soils. This permits using nitrification as a test for soil pollution by pesticides (Grishina and Morgun, 1986).

1.7 EFFECTS OF PESTICIDES ON DENITRIFICATION

Loss of gaseous nitrogen by denitrification is undesirable in terms of soil fertility, so pesticides affecting this process could be considered beneficial.

The effects of 20 commonly used herbicides on denitrification of nitrate in three soils were studied by determining the effects of 10 and 50 $\mu g\ g^{-1}$ soil of each herbicide on the amounts of nitrate lost and the amounts of nitrite, N_2O and N_2 produced when soil samples were incubated anaerobically after treatment with nitrate. The herbicides used were butylate, EPTC (ethyl dipropyl thiolcarbamate), chlorpropham, propham, diuron, linuron, monuron, siduron, alachlor, trifluralin, 2,4-D amine, 2,4-D ester, atrazine, cyanazine, metribuzin, simazine, dalapon, chloramben, dicamba and dinoseb. None of the herbicides studied significantly affected denitrification of nitrate when applied at the rate of 10 $\mu g\ g^{-1}$ soil, but dinoseb increased the ratio of N_2 to N_2O in the gaseous products of denitrification when applied at this rate. Butylate, EPTC, diuron, simazine and dalapon had no significant effect on denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ soil, whereas metribuzin and dineseb enhanced denitrification when applied at this rate. The influence of the other herbicides on denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ depended on the soil, but all enhanced or inhibited denitrification in at least one soil (Yeomans and

Bremner, 1985).

In further studies Bremner and Yeomans (1986) using the same 20 herbicides found that none of the tested herbicides inhibited denitrification when applied at the rate of 10 $\mu g\ g^{-1}$ soil, but dinoseb increased the ratio of N_2 to N_2O in the gaseous products of denitrification. Dinoseb and metribuzin enhanced denitrification in the three soils used when they were applied at the rate of 50 $\mu g\ g^{-1}$ soil. Dinoseb also increased the ratio of N_2/N_2O in the gaseous products of denitrification when applied at this rate and metribuzin inhibited nitrite reduction in the Storden soil when applied at this rate of 50 $\mu g\ g^{-1}$ soil.

Six of the herbicides (chlorpropham, propham, alachlor, 2,4-D amine, 2,4-D ester, and dicamba) either had no appreciable effect on denitrification, or enhanced denitrification, when applied at the rate of 50 $\mu g\ g^{-1}$ soil, and all six of these herbicides increased the ratio of N_2/N_2O in the gaseous products of denitrification when applied at this rate (Bremner and Yeomans, 1986).

Denitrifying bacterial populations in soil have been reported to be enhanced by 2,4-D (Sethunathan, 1970). Denitrification by cultures of denitrifying bacteria has been reported to be inhibited by MCPA (2-methyl-4-chlorophenoxyacetic acid), 2,4,5-T (Tortensson, 1974), 2,4-D, chlorbromuron, chloroxuron, fluometuron, metoxuron, neburon, diuron, linuron, siduron (Hart and

Larson, 1966; Bollag and Nash, 1974; Bollag and Henniger, 1976), and dalapon (Grant and Payne, 1982), to be enhanced by MCPA (2-methyl-4-chlorophenoxyacetic acid), 2,4,5-T, linuron and simazine (Torstensson, 1974) and to be unaffected by atrazine and simazine (Bollag and Henninger, 1976). Other herbicides found to have no effect on denitrification by cultures of denitrifying bacteria include fenuron, metobromuron, monuron, ametryne, endothal, hydroxysimazine, and propham (Bollag and Nash, 1974; Bollag and Henninger, 1976).

Metolachlor has been found to inhibit denitrification in soil (Bollag and Kurek, 1980). Eight herbicides reported to inhibit denitrification by cultures of denitrifying bacteria have also been reported to inhibit denitrification in soil. These are 2,4-D (Bollag and Henninger, 1976), fluometuron (Bollag and Kurek, 1980), dalapon (Weeraratna, 1980), diuron, linuron, neburon, simazine and atrazine (Yeomans and Bremner, 1985). Atrazine has also been reported to enhance denitrification in soil (Cervelli and Rolston, 1983) and to have no effect on denitrification in soil (Bollag and Henninger, 1976). Other herbicides that appear to have no effect on denitrification in soil are diuron, linuron, simazine, ametryne, endothal, hydroxysimazine, propham and siduron (Bollag and Henninger, 1976).

Bollag and Nash (1974) concluded that inhibition by phenylurea and aniline herbicides depended upon the

number of halogen substituents on the aromatic ring.

In reviewing earlier work, Anderson (1978) noted that most dithiocarbamate fungicides are inhibitory while alkyl-diamino-dithiocarbamates, such as nabam and maneb, are more toxic than their dialkylamine counterparts ferbam, thiram or ziram. In this latter group the inhibitory effect is proportional to the number of dithiocarbamate radicals. Thus, ferbam is more toxic than thiram and the latter more so than ziram.

Bremner and Yeomans (1986) studied the effects of six fungicides on denitrification of nitrate in soil and found that none of the fungicides studied had a significant effect when applied at the rate of 10 $\mu g\ g^{-1}$ soil, and only captan inhibited denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ soil.

Captan promoted reduction of N_2O to N_2 in the Harps soil when applied at the rate of 10 $\mu g\ g^{-1}$ soil, but it retarded NO_2 reduction in the Storden soil when applied at this rate and maneb inhibited nitrite reduction in the Storden soil when applied at the rate of 10 $\mu g\ g^{-1}$ soil. Three of the fungicides, maneb, thiram, and terrazole either had no appreciable effect on denitrification, or enhanced denitrification when applied at the rate of 50 $\mu g\ g^{-1}$ soil, and these three fungicides increased the ration of N_2/N_2O in the gaseous products of denitrification when applied at this rate (Bremner and Yeoman, 1986).

In sandy loam, clay loam and clay soils, insecticide chlordane or endrin at the rate of 22 kg ha^{-1} and fonofos or pirimiphos-ethyl at 11 kg ha^{-1} increased denitrification (Gawaad et al., 1972b, 1973b). Bollag and Henninger (1976) found that carbofuran had no effect on denitrification when applied at the rate of 100 μg g^{-1}.

Lindane and malathion enhanced denitrification in three soils when they were applied at the rate of 50 μg g^{-1} soil while fenitrothion, phorate, terbufos and carbofuran either had no appreciable effect on denitrification or enhanced denitrification when applied at this rate. Fenitrothion, phorate, terbufos, and carbofuran increased the ratio of N_2/N_2O in the gaseous products of denitrification when applied at the rate of 50 μg g^{-1} soil, (Bremner and Yeomans, 1986). Grant and Payne (1982) reported that malathion inhibited denitrification when applied at the rate of 1250 μg g^{-1} sediment to a salt marsh sediment.

Very little is known about the effects of herbicides, fungicides, and insecticides on denitrification of nitrate in soil. Moreover, the limited studies that have been reported concerning the effects of pesticides on denitrification of nitrate by soil microorganisms have given divergent results, and the methodology used in most of these studies is open to criticism (Bremner and Yeomans, 1986).

1.8 EFFECTS OF PESTICIDES ON NITROGEN FIXATION

Nitrogen fixation by symbiotic organisms associated with legumes is of unarguable importance throughout the world. The microorganisms involved in fixing N_2 are either free living and nonsymbiotic such as the blue-green algae, Azotobacter spp. and Clostridium pasteurianum, or like Rhizobium spp., exist in symbiosis with a higher plant. The blue-green algae make the greatest contribution of soil fertility, especially in lowland rice cultivation, by fixing atmospheric nitrogen (Edwards, 1989).

Most herbicides do not affect the growth of aerobic nonsymbiotic nitrogen-fixing bacteria when they are used at the recommended doses (Edwards, 1989). Many workers report that herbicides have relatively little effect on Azotobacter at the recommended field application rates. However, very large doses can affect the growth and nitrogen fixation by this organism (Grossbard, 1976).

Babak (1968) investigated ten Azotobacter strains and found halophilic strains to be inhibited by 1000 ppm 2,4-DES [2-(2,4-dichlorophenoxy)ethyl hydrogen sulphate] and 2,4-DEP but mesophilic strains required 2000 to 3000 ppm of these herbicides for inhibition to occur. Similarly, while 5000 to 6000 ppm chlorazine, DCNB (dichloronitrobenzene) or diphenamid inhibited halophilic Azotobacter sp. strains, mesophilic strains needed 6000 to 7000 ppm.

A few herbicides have been reported to have a depressive effect on Azotobacter populations under certain conditions and at rates not much above the recommended field application rates. These include linuron, monolinuron, and cycluron + chlorbufam (Edwards, 1989) and dinoseb acetate (Simon-Sylvestre and Fournier, 1979). Dinoseb inhibited reduction of C_2H_2, indicating effects on nitrogen fixation (Vlassak et al., 1976). In a study of the effects 32 pesticides on C_2H_2 reduction, only ethoprop had effect, even at high doses (Tu, 1978).

Other herbicides have stimulating effects on population of nonsymbiotic nitrogen-fixing bacteria such as Azotobacter, including simazine, atrazine, trietazine, prometryn and MCPA (2-methyl-4-chlorophenoxyacetic acid) (Edwards, 1989). Few workers reported stimulatory effects of herbicides on Azotobacter, these are 2,4-D (Sharma and Saxena, 1974), and atrazine (Simon-Sylvestre and Fournier, 1979).

A few reports indicate some adverse effects of herbicides on nitrogen fixation such as linuron, chloropropham and dalapon (Edwards, 1989); ioxynil, mecoprop, dalapon and dichlorprop (Tu and Bollen, 1968); and prometryn (Grossbard, 1974).

Clostridium pasteurianum can fix nitrogen under anaerobic conditions but the effects of herbicides on this organism have been studied very little. The few reports in the literature indicate that herbicides have

very little effect on *Clostridium*. Overall there is very little evidence of any important effects on herbicides on nonsymbiotic nitrogen-fixing bacteria (Edwards, 1989).

Symbiotic nitrogen fixation by *Rhizobium* is the most efficient nitrogen fixation process. The response of *Rhizobium* to herbicides is of two types, one on the bacteria itself and its growth, and other on the host plant, in terms of infestation, the phenomenon of root nodule formation, and nitrogen fixation. Although there are a few reports of the effects of herbicides on *Rhizobium*, there is little evidence of any practical effects on nitrogen fixation. In a survey of the effects of 104 herbicides, including 26 strains from 6 species, it was reported that only 6 herbicides inhibited *Rhizobium*. These were dinoseb acetate, a mixture of ioxynil and mecoprop, dazomet, diallate, N-butyl-N-ethyl-2,6-dinitro-4-trifluormethyl-aniline, and MCPB [4-(4-chloro-2-methylphenoxy)butyric acid] (Edwards, 1989).

The selectivity of herbicides with respect to their effects on different species of *Rhizobium* is very pronounced (Grossbard, 1976). The most inhibitory herbicides are DNOC (2-methyl-4,6-dinitrophenol) and dinoseb (Grossbard, 1976); and linuron (Grossbard, 1975).

It has been reported that nodulation and nitrogen fixation in soils were reduced by atrazine, dinoseb,

asulam, and linuron (Garcia and Jordan, 1969). Edwards (1989) reported that the decrease in nodulation and nitrogen fixation was the result of damage caused by the herbicide to the plants and abnormal growth of the roots. The evidence that herbicides significantly affect nitrogen fixation in terms of soil fertility is sparse.

Nine fungicides, at 4, 40 and 400 ppm, had no effect on Azotobacter chroococuum cultures, but copper oxychloride at 40 and 400 ppm completely inhibited growth. Pure cultures of Azotobacter chroococcum were inhibited by zineb (5000 and 50000 ppm), captan (3000 and 30000 ppm), ferbam (3000 and 30000 ppm), thiram (5000 ppm), folpet (30000 ppm) and maneb (30000 ppm), while an antibiotic mixture, Fungicidin, had no effect at 5000 ppm (Anderson, 1978). Benomyl (2 kg ha^{-1}) had no effect on Azotobacter spp. in sandy soil (van Fassen, 1974). But captan applied at field rates to cotton cultivators inhibited Azotobacter spp. in the rhizosphere soil (Mahmoud et al., 1972).

Anderson (1978) reviewed that at the lowest concentration (0.03 ppm) captan, thiram, Ceresan, dichlone and methylarsinic sulphide were toxic to some strains of Rhizobium, whereas in the range 300 to 30000 ppm most strains were inhibited. In an experiment with 33 fungicides and 21 strains of Rhizobium leguminosarum showed that captan, thiram, dichlone and chloranil were moderately inhibitory whereas Ceresan and MMDD

(methylmercury dicyandiamide) were strongly inhibitory. Fisher and Clifton (1976) found that benomyl, triforine and tridemorph had little effect on strains of _Rhizobium leguminosarum_ and _Rhizobium meliloti_ with concentrations of up to 200 ppm in agar medium of pH 5.5 to 7.5. Using purest grade unformulated fungicides, Fisher (1976) showed that growth of _Rhizobium trifolii_ in agar medium with 10, 50, 100 and 200 ppm each of captan, dodine and oxycarboxin was markedly decreased.

An octylphenol/ethylene oxide condensate also decreased growth but benomyl, carboxin, dimethirimol, ethirimol, tridemorph and thiophanate-methyl had little effect even at highest concentrations. Triforine at 100 or 200 ppm was slightly inhibitory, but not at 10 or 50 ppm. In liquid culture at rates of 10-1000 ppm the inhibitory effects of captan, dodine and oxycarboxin were confirmed (Fisher, 1976).

Staphorst and Strijdom (1976) investigated the effects of 13 fungicides on _Rhizobium_ strains capable of nodulating _Vigna anguiculata_. Of 13 fungicides, quintozene, benomyl, fenaminosulf and 2-hydroxypropyl methanethiosulphonate were least toxic and a mixture of diclroan and captafol, captan, and a mixture of captan with phenylmercuryacetate were intermediate in their effect, while carboxin, thiram, mancozeb, maneb and TCMTB [2-(thiocyanomethylthio)benzothiazole] were the most toxic.

Using sterile and non-sterile soil, Fisher (1976) found that with root drenches of dodine, captan, carbendazim, Ethylan CP, thiram and ethirimol, at rates equivalent to 25 and 50 kg ha^{-1} in each case, the dry weight and nitrogen content of Rhizobium trifolii-inoculated white clover plants were not affected. Only three of the fungicides had any effect on N-fixation in excised nodules. Ethylan CP at 25 kg ha^{-1} increased nitrogen fixation but at 50 kg ha^{-1} had no effect. Thiram at 50 kg ha^{-1} increased nitrogen fixation significantly. Oxycarboxin at rates equivalent to 2.5, 5, and 15 kg ha^{-1} significantly decreased N-fixation, whereas ethirimol did not.

Fungicides appear to differ in their effects on growth of Rhizobium depending on the strain of Rhizobium involved. Daitloff (1970) has suggested that toxic effects of fungicides may be prevented by coating legume seed with a layer of polyvinylacetate after fungicide-treatment but before inoculation with Rhizobium.

Insecticides in general do not seem to inhibit nonsymbiotic, nitrogen fixing microorganisms in soil. Salem and Gulyas (1971) found that lindane (10 ppm), fonofos (50 ppm) and diazinon (50 ppm) initially inhibited glucose utilization and N_2-fixation in Azotobacter chroococcum and Azotobacter agile but later these functions increased. Lindane and DDT, at the

equivalent of field rate and 50 times field rate, had no significant effect on N_2-fixation in Azotobacter vinelandii (Anderson, 1978).

In clay soil treated with 0.22 and 4.4 kg ha^{-1} of lindane or dieldrin at 2.5 and 50 kg ha^{-1}, population numbers of Azotobacter increased, but lindane decreased and dieldrin increased the numbers of anaerobic N_2-fixers (Mahmoud et al., 1970). Parathion-methyl at 0.15 kg a.i. ha^{-1} briefly increased population numbers of N_2-fixing Azotobacter in a loam soil and higher rates (15, 150 and 300 kg a.i. ha^{-1}) also increased population numbers, but only after a transient decrease (Naumann, 1970a).
Several strains of symbiotic Rhizobium have been found to be resistant to insecticides, some are stimulated while others are sensitive. Kapusta and Rouwenhorst (1973) tested the interaction of ten insecticides and Rhizobium japonicum strains in pure culture and found that only disulfoton, at 7.5 ppm, inhibited a mixture of strains while the other insecticides had no effect. When screening individual strains, these workers observed different sensitivities of six strains to disulfoton and carbaryl. Disulfoton reduced growth of all strains and only two strains successfully grew at the highest rate (24 ppm) while with carbaryl, two strains were completely inhibited by the highest concentration (40 ppm) and one strain was resistant. Salem (1971) found that lindane and fonofos at 10 and 50 ppm, respectively, inhibited

glucose utilization of an effective strain of Rhizobium trifolii and increased it in an ineffective strain; conversely, diazinon (50 ppm) increased glucose consumption in the effective strain.

A group of insecticides were tested for inhibition of Rhizobium on agar medium and the most sensitive and similar in all cases were Rhizobium trifolii and Rhizobium leguminosarum (Shin-Chsiang Lin et al., 1972). Increasing concentrations of disulfoton, carbofuran and endrin inhibited Rhizobium sp. (Oblisami et al., 1973). Dimethoate at the rate of 1.89 ppm allowed normal growth, whereas at the rate of 3.78 ppm completely inhibited two strains of Rhizobium meliloti (Staphorst and Strijdom, 1974).

Treatment of Medicago sativa seed with dimethoate at the rates of 37.8 to 226.8 ml a.i. 50 kg^{-1} seed five days before inoculation with two strains of Rhizobium meliloti decreased plant yield in quartz sand culture but did not affect nodulation of the plants, except at the higher rate. When seed was treated with 113.4 ml a.i. 50 kg^{-1} seed and then inoculated two to nine days later, the yield of plants was better than those inoculated immediately after treatment. Dimethoate, at 0.3 ppm a.i. as foliar spray applied to inoculated and non-inoculated lucerne, had no significant effect on nodulation but sprayed plants were smaller than those not sprayed (Staphorst and Strijdom, 1974).

Carbofuran at the rate of 16 kg ha^{-1} had no effect whereas phorate at 8 kg ha^{-1}, fensulfothion at 20 kg ha^{-1} and heptachlor at 18 kg ha^{-1} each increased the size but decreased the numbers of nodules on plants in a loam soil. None of these compounds affected the plant weight, yield, leghaemoglobin or nitrogen content (Kulkarni et al., 1974).

Under laboratory conditions, lindane, DDT, heptachlor and aldicarb significantly reduced, while phorate increased numbers of nodules per plant on bean and clover, whereas under field conditions at higher application rates phorate, aldicarb, and lindane did not affect nodulation of clover roots (Gawaad et al., 1972a). Lindane at the rates of 5, 50 and 500 kg ha^{-1} in an alluvila soil, decreased nodulation and efficiency of nodules (Mishra and Gaur, 1975). Overall insecticides have a general inhibition of symbiotic, N_2-fixing organism and nodulation (Anderson, 1978).

1.9 EFFECTS OF PESTICIDES ON TRANSFORMATIONS OF OTHER ELEMENTS

Much less emphasis has been placed on the mineralization of other elements such as phosphorus and sulphur. In most mineral soils, one half to two thirds of the total phosphorus present is inorganic forms so it is an important source of plant and microbial nutrients. There are several methods of following mineralization of

organic phosphorus (Anderson, 1960; Greaves and Malkomes, 1980). The most suitable methods for assessing the effects of herbicides, as with those used for nitrogen transformations, involve treatment and then simple soil incubations accompanied by extraction and measurement of inorganic phosphorus at suitable intervals. Such methods will probably only detect major changes in mineralization, but it is only such changes which are likely to be of significance to plant nutrition (Edwards, 1989).

Only a few workers mention the effects of pesticides on the bacteria of the phosphorus cycle. Among the herbicides, simazine and 2,4-D reduce the growth of these bacteria (Yurkevich and Tolkachev, 1972), but trifluralin has no effect on phosphorus mineralization (Simon-Sylvestre and Fournier, 1979).

The organophosphorus insecticides do not affect the mineralization of the organic phosphorus of the soil (Tu, 1970). Ogunseitan and Odeyemi (1985) found that the rate of phosphate solubilization was decreased by 50% by captan in 15 days, whereas in lindane and malathion-treated soils, the rates were decreased to 34 and 3%, respectively, of the normal rate.

Phosphorus mineralization is stimulated after a fungicide treatment with thiram (Agnihotri, 1974).

In soil, transformation of sulphur, an element essential to all living cells, seems to be mainly a

microbial process. Very few studies have been carried out on the behaviour of the sulphur-oxidizing bacteria in response to herbicides (Simon-Sylvestre and Fournier, 1979). However, sulphur oxidation is not affected by 2,4-D, MCPA (2-methyl-4-chlorophenoxyacetic acid), maleic hydrazide, or ammonium sulfamate, even at concentrations well above field rates (Simon-Sylvestre and Fournier, 1979). Paraquat, on the other hand, has a slightly harmful action (Tu and Bollen, 1968).

The insecticides, in general, have no effect on sulphur-oxidizing bacteria. This has been shown with acephate and methamidophos, even at concentrations ten times the normal rate (Focht and Josseph, 1974). However, an application of 100 $\mu g/g$ of diazinon leads to an increase in sulphur oxidation of about 15%, and thionazin and chlorpyrifos, at same concentration, cause a decrease ranging from 12 to 17% (Tu, 1970).

Ray and Sethunathan (1980) reported that the addition of commercial formulations of HCH an insecticide and benomyl a fungicide in alluvial and lateritic soils, inhibited sulphur oxidation in both soils, the inhibition being more pronounced in alluvial soil. Also, HCH was more inhibitory than benomyl.

Malathion at the rate of 50 mg kg^{-1} increased the rate of sulphur oxidation in soil by 8% and lindane and captan at the rate of 100 mg kg^{-1} each, affected this reaction adversely by 10 and 3%, respectively (Ogunseitan

and Odeyemi, 1985).

Elementary sulphur oxidation is slower with some nematicides, such as fensulfothion, methylisothiocyanate, DD, and Carbofuran (Tu, 1972).

Until more is known of the nature of sulphur-containing organic matter, and the conditions affecting its stability, it seems reasonable to conclude that sulphur mineralization is not a suitable function to study in relation to pesticides effects (Greaves and Malkomes, 1980).

Similar conclusions can be drawn concerning elements such as calcium, magnesium, sodium, silicon and chlorine. Although all these elements contribute to the fertility of soil, it is not yet feasible to include studies of pesticide effects of their interrelationships with soil microorganisms as part of a regulatory package (Greaves and Malkomes, 1980; Edwards, 1989).

1.10 EFFECTS OF PESTICIDES ON UREASE ACTIVITY IN SOIL

The use of nitrogen fertilizers has steadily increased during the past 20 years throughout the world, and the greatest percentage increase has been in the use of urea. Urea is the main form of nitrogen used in developing countries, and its use is still significantly increasing. The urea is rapidly hydrolyzed under favourable conditions to ammonium bicarbonate, which is subsequently nitrified. Nitrite and nitrate ions and

urea can readily diffuse through the soil or be leached. However, ammonium ions are much less mobile (Gould et al., 1986).

The enzyme urease which catalyzes the hydrolysis of urea is found in some higher plants and in most species of bacteria, yeast and fungi. The ability to hydrolyze urea was found to vary from 17 to 71% for soil bacteria and from 78 to 98% for soil fungi (Lloyd and Sheaffe, 1973). Although soil urease is considered to be of microbial origin there is evidence that some soil urease activity may be derived from plants (Frankenberger and Tabatabai, 1982).

The currently accepted reaction mechanism for the enzymatic hydrolysis of urea is the enzymatic cleavage of urea to ammonia and carbamic acid followed by the chemical hydrolysis of carbamic acid to ammonia and carbon dioxide (Blakeley et al., 1982).

$$H_2N{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}NH_2 \;+\; \text{urease} \;\longrightarrow\; \text{urease}(H_2N{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}NH_2)$$

$$\text{urease}(H_2N{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}NH_2) \;\underset{}{\overset{+\,H_2O}{\rightleftharpoons}}\; NH_3 + H_2N{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}OH + \text{urease}$$

$$H_2N{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}OH \;\xrightarrow{+\,H_2O}\; NH_3 + CO_2$$

Studies have showed that several compounds can retard the conversion of urea to ammonium by soil urease.

Studies to evaluate more than 100 compounds as inhibitors of urease activity in soils showed that dihydric phenols and quinones were the most effective organic compounds tested and that silver and mercury salts were the most effective inorganic compounds (Bremner and Douglas, 1971).

Urease inhibitors can retard the rapid biological hydrolysis of urea and they have received increasing attention recently (Hendrickson et al., 1987; Schlegel et al., 1987). However, only a few studies mention the effects of these compounds on the conversion of ammonium to nitrite and nitrite to nitrate by the nitrifying microorganisms (Bundy and Bremner, 1974; Mishra, 1980).

Bremner and Douglas (1973) re-examined 8 of the most effective inhibitors of soil urease activity. The results showed that, of the inhibitors re-examined, 2,5-dimethyl-p-benzoquinone had the greatest ability, and AHA the least ability to retard urea hydrolysis in soils and reduce gaseous losses of urea N as ammonia. The effects of compounds differed markedly for different soils and were greatest with a sandy soil and least with a clay loam.

Mishra (1980) studied the effect of quinoid and phenolic compounds on urease activity in brown earth and black earth soils and found that all chemicals used inhibited urease activity at a concentration of 20 ppm in both soils.

Zhenping et al. (1990) found that addition of phenylphosphorodiamidate (PPDA) or N-bytylphosphoro-thionic triamide (NBPT) did not influence the oxidation of ammonium. Hydroquinone (HQ), however, retarded the process significantly, and also the accumulation of nitrite.

Herbicides that have been reported to decrease urease activity in soils include trifluralin and 2,4,5-T at the rate of 300 kg ha^{-1} in calcareous loam soil; pyrazon at 4 to 8 kg ha^{-1} and atrazine at 1 kg ha^{-1} in silty medium loam derno-podzol; dalapon, 2,4-D, and methurin at the rate of 20, 2 and 3 kg ha^{-1} respectively in silty medium loam derno-podzol (Anderson, 1978); and atrazine at 4 kg ha^{-1} applied to a loamy sand (Voets et al., 1974).

Prometryn, linuron, and monolinuron at the rate of 10 to 100 kg ha^{-1} in sandy and loamy soil inhibited the urease activity (Anderson, 1978). Inhibition of soil urease by 0.1M MCPB [4-(4-chloro-2-methylphenoxy)butyric acid] and 2,4-D were 9.8 and 2.1% respectively (D'Arrigo and Ioppolo, 1989).

Some herbicides which do not affect the urease activity include monilate at the rate of 0.75 to 1.5 kg ha^{-1} and 2,4-D at 0.4 kg ha^{-1} in pale sandy loam chernozem soil (Chulakov and Zharasov, 1973); 2,4-D at 2 and 100 kg ha^{-1}, and simazine at 4 and 200 kg ha^{-1} in chernozem soil (Yurkevich and Tolkachev, 1972); and simazine at 2 kg ha^{-1} applied to chernozen medium clay

(Kulinska, 1967). Four herbicides 2,4-D, diallate, benzoylprop-ethyl and glyphosphate at concentrations equivalent to five times the recommended field application rates had no effect on the acivity of urease in soil (Lethbridge et al., 1981).

Other herbicides which have been reported to stimulate urease activity in soils include dichlobenil at the rate of 2.8 to 13 kg ha^{-1} applied to 3.2% o.m. shale, dinoseb at 5 kg ha^{-1} in silty medium loam derno-podzol; atrazine at 1-4 kg ha^{-1} and paraquat + diquat in calcareous and shale soils; simazine at 5 kg ha^{-1} in silty medium loam derno-podzol (Anderson, 1978); and methurin at 4 kg ha^{-1} in derno-podzolic sandy loam and medium loam (Kruglov et al., 1973). Dalapon at 10 kg ha^{-1} and paraquat at 3.75 kg a.i. ha^{-1} increased urease activity by 67-79% applied to grassland oxison in combination with urea or separately (Namdeo and Dube, 1973). Linuron and simetryn applied to a loam soil also increased urease activity (Kim et al., 1988).

Urease activity was stimulated at concentration of 20 ppm by the fungicide dexon, but inhibited at 100 and 200 ppm concentrations. The inhibition was found to be related to the fungicide concentration and varied with incubation time (Karanth et al., 1975). 2-(thiocyano-methylthio)benzothiazole (TCMTB) at the rate of 5 to 30 kg ha^{-1} in an arable soil inhibited urease activity in proportion to the concentration of the compound (Voets

and Vandamme, 1970).

Tsirkov (1970) reported that insecticides hexachlordane and heptachlor decreased urease activity in meadow soils whereas lindane and dieldrin increased the activity of this enzyme. Similarly, lindane, aldrin and heptachlor did not prevent enzymic hydrolysis of urea in arable or meadow soils (Verstraeten and Vlassak, 1973). Malathion at concentrations equivalent to five times the recommended field application rate had no effect on the activity of urease enzyme (Lethbridge et al., 1981). Three organophosphorus insecticides accothion, malathion and thimet at the rates of 50, 200 and 1000 ppm, inhibited soil urease activity (Lethbridge and Burns, 1976).

Thus a wide variety of chemicals have been found to affect the activity of the enzyme, urease including certain organochlorine insecticides (Tsirkov, 1970), substituted urea herbicides (Cervelli et al., 1975, 1976, 1977), organo-phosphorus insecticides (Lethbridge and Burns, 1976). Polyphenols cause some degree of inhibition (Fernando and Roberts, 1976) as do several heterocyclic mercaptans (Gould et al., 1978). Mercuric ion evidently is the most inhibitory of all chemicals tested (Olson and Christensen, 1982).

Because of the sensitivity of urease to many foreign chemicals, it is a useful indicator for the presence of certain reactive chemicals in field situations, where,

for example, it has been analyzed in soil biota to reflect the presence of fertilizers, herbicides, insecticides and environmental pollutants (Tyler, 1974; Mishra and Flaig, 1979). This enzyme in the immobilized state was also used for the determination of the presence in the environment of trace amounts of heavy metal ions such as mercury (Ogren and Johannson, 1978).

1.11 EFFECTS OF PESTICIDES ON PHOSPHATASE ACTIVITY IN SOIL

The general name phosphatase has been used to describe a broad group of enzymes that catalyse the hydrolysis of both esters and anhydrides of H_3PO_4 (Schmidt and Laskowski, 1961).

Because of the importance of these enzymes in soil organic P mineralization and plant nutrition, considerable literature has accumulated on phosphomonoesterases in soils (Speir and Ross, 1978). Most of the literature, however, is related to acid phosphatase. Consequently this enzyme has been given a prominent place in a number of soil biochemistry and enzymology reviews (Cosgrove, 1967; Kiss et al., 1975; Speir and Ross, 1978).

Of several herbicides applied to sugar beet, pyrazon caused the greatest inhibition of phosphatase activity in soil (Voets and Verstraete, 1973). Simazine at the rate of 200 kg ha^{-1} applied to chernozem soil also inhibited

phosphatase activity (Yurkevich and Tolkachev, 1972). Other herbicides that have been reported to decrease the phosphatase activity are 2,4-D at 2 and 100 kg ha^{-1} in chernozem soil (Yurkevich and Tolkachev, 1972); atrazine at 4 kg ha^{-1} in loamy sand (Voets et al., 1974); and lenacil at 1 kg ha^{-1} + DCU (dichloral urea) at 5 kg ha^{-1} in chernozem soil (Barona, 1974).

Atrazine at the rate of 2 and 10 kg ha^{-1} applied to a podzolized medium loam chernozem soil, slightly affected the phosphatase activity in first year but increased in second year (Manorik and Malickenko, 1969).

Some herbicides do not affect the phosphatase activity. These include 2,4-D at the rate of 0.4 kg ha^{-1} in pale sandy loam chernozem soil and lenacil at 1.2 to 1.7 kg ha^{-1} in dark chestnut heavy loam and pale chestnut light loam soil (Chulakov and Zharasov, 1973); and atrazine at the rate of 4 and 10 kg ha^{-1} in heavy loam chernozem soil (Pilipets and Litvinov, 1972).

Simazine at the rate of 4 kg ha^{-1} applied to a chernozem soil increased the phosphatase activity (Yurkevich and Tolkachev, 1972). Boiko et al. (1969) reported that simazine at the rate of 2 kg ha^{-1} also increased the phosphatase activity when applied to a grey forest silty medium loam soil. Similarly, diuron at the rate of 6 kg ha^{-1} and simazine at 6 to 12 kg ha^{-1} in leached loam chernozem soil increased the phosphatase activity (Arshidinov et al., 1974).

Fungicide TCMTB [2-thiocyanomethylthio)benzothiazole] at the rate of 5 to 30 kg ha^{-1} applied to an arable soil inhibited phosphatase activity in proportion to the concentration of the compound (Voets and Vandamme, 1970).

In a study of the effects of organophosphorus and carbamate insecticides on enzymic activities in the soil environment Hong and Kim (1986) found that the inhibitory effects of the insecticides on enzyme activities in soil decreased in the following order: dithiophosphoric acid > thiophosphoric acid > phosphoric acid > carbamate insecticides for phosphatase enzyme.

The concentration-dependant effect of organo-phosphorus, organochlorine and synthetic pyrethroids on soil acid phosphatase was studied under laboratory conditions. At 0.02 mol the percentage of inhibition was as follows: malathion (96%) > fenitrothion ~ permethrin > monocrotophos > chlorpyrifos > chlordane > aldrin > lindane > bioresmethrin > mephosfolam (15%) (Komeil, 1986).

Effect of 35 compounds on enzyme activities in an organic soil was studied by Tu (1981) and found that phosphatase activity was reduced by most treatments, whereas chlordane at 5 $\mu g\ g^{-1}$ increased activity.

Sikora et al. (1990) in a study to determine whether soils that showed enhanced biodegradation of organophosphate insecticides had significantly different

enzyme activities from those in the same soils with no previous exposure to the insecticides. Twenty-one pairs of soils were collected from farms in the Midwest where Chlorpyrifos, terbufos, fonofos, or phorate had failed to protect corn (*Zea mays*) from corn root worm. Each soil was analysed for several enzyme activities including acid and alkaline phosphatase. Over 40% of the insecticide-treated soils had higher acid phosphatase activity then the fence row soils which had no previous exposure to the insecticide. Over two-thirds of the soils treated with fonofos had higher acid phosphatase activity then the fence row soils.

A laboratory study was conducted to determine the effect of four experimental insecticides DOWCO 429X, DPX 43898, tefluthrin, and trimethacarb, on enzyme activities in mineral and organic soils. Phosphatase activity in insecticide treated samples was equal to or greater than that of control in sandy soil after 2 hours. With the exception of DPX 43898, the insecticides depressed phosphatase activity in most organic soil samples (Tu, 1990).

1.12 EFFECT OF PESTICIDES ON DEHYDROGENASE ACTIVITY IN SOIL

The dehydrogenase enzyme systems apparently fulfil a significant role in oxidation of soil organic matter because they transfer hydrogen from substrates to

acceptors. Many different specific dehydrogenase systems are involved in the dehydrogenase activity of soils; these systems are an integral part of the microorganism. Therefore, the result of the assay of dehydrogenase activity would show the average activity of the active population (Skujins, 1976).

In a laboratory experiment Hickisch et al. (1984) reported that amending loam samples 5 times at 2-week intervals with rates of tribunil 3 kg ha^{-1}, trichloroacetal dehydrate 20 to 50 litre ha^{-1} and wolfen-thiuram 2 kg ha^{-1}, all three herbicides severely inhibited soil dehydrogenase activity.

Avadex and Ro-Neet (cycloate) were applied alone or in combination with a complete pesticide treatment system to sugar beet. During the growing season, soil samples were taken from 0-5 and 5-10 cm soil layers - and dehydrogenase activity was determined. Avadex alone caused minor inhibition of the dehydrogenase activity in 0-5 cm soil layer, whereas in 5-10 cm depth a marked decrease was observed in first year. Ro-Neet alone hardly caused inhibitions of the enzyme activity in both soil layers (Malkomes, 1982). Roundup at recommended and excessive rates, decreased dehydrogenase activity by 0-10% (Schuster et al., 1989).

Many other herbicides have been reported to decrease dehydrogenase activity in soil. These include chlorthiamid at the rate of 15 to 30 kg ha^{-1} and atrazine

+ mecoprop in limestone soil; chlorthiamid at 15 to 30 kg ha^{-1} in calcareous and shale soils; linuron at 1-10 kg ha^{-1} in sandy loam soil (Anderson, 1978) and sodium chlorate at 150 kg ha^{-1} in turfed plots of acid sandy soil (Karki et al., 1973). Atrazine, fluometron, and trifluralin were tested as soil dehydrogenase inhibitors and atrazine was found far more toxic to dehydrogenase than fluometuron and trifluralin (El-Din et al., 1977). Chloroxuron at the rate of 1-10 kg ha^{-1} applied to three clay soils did not affect the dehydrogenase activity (Odu and Horsfall, 1971).

Addition of fungicide benomyl at 1 to 100 $\mu g/g$, at flooding, prevented dehydrogenase accumulation (Chendrayan and Sethunathan, 1980). Fenaminosulf also decreased dehydrogenase activity (Karanth and Vasantharajan, 1973). Captan in concentrations of 250ppm first stimulated dehydrogenase activity but after 16 weeks the activity was identical with that in untreated soil (Naumann, 1970b).

In a laboratory experiment to determine the effect of four experimental insecticides on enzyme activities in mineral and organic soils Tu (1990) found that none of the insecticide treatments inhibited dehydrogenase activity in either soil. Dehydrogenase activity was greater in many samples in sandy loam soil than the control throughout the experiment. At rate-equivalents of 15 kg a.i. ha^{-1} or lower, parathion methyl increased

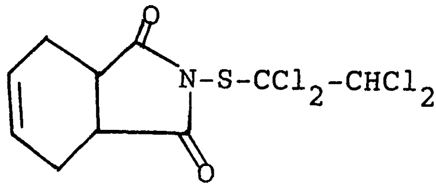
dehydrogenase activity in soil but higher rates of 150 and 300 kg a.i. ha^{-1} completely inhibited the enzynme activity (Naumann, 1970a).

Dehydrogenase was the most sensitive and so could be useful test for the side effects of pesticides on soil microorganisms (Auspurg et al., 1989; Malkames, 1991).

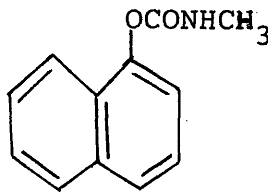
1.13 AIMS OF THE THESIS

The aim of the work described in this thesis is to study the effects of pesticides on soil microorganisms and their activities.

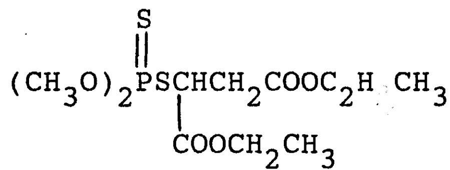
Three commonly used pesticides were chosen. Firstly captafol which is a non-systemic fungicide widely used to control foliage and fruit disease of tomatoes and potatoes from blight. It is practically insoluble in water but slightly soluble in most organic solvents. It has a non specific mode of action that can be illustrated by the breadth of chemical effects. These include the inhibition of thiolenzymes, interactions with membranes and disruption of mitochondrial reactions including oxidative NADH oxidation.



Captafol (dicarboximide)



Carbaryl (carbamate)



Malathion (dithiophosphate)

Chemical formulae of pesticides used

The second pesticide chosen was carbaryl. It is a contact and stomach insecticide with slight systemic properties. It is recommended for use against many insect pests of cotton, fruit, vegetables and other crops. It is also used to reduce the number of fruits on heavily laden apple trees. Its solubility in water at 30°C is 40 ppm and it is soluble in most polar organic solvents. Carbaryl is hydrolysed at the ester linkage by an enzyme present in albumin fraction of plasma.

The third one was malathion which is a nonsystemic organophosphorus insecticide and acaricide of low mammalian toxicity. Hydrolysis in most vertebrates leads to detoxication but rapid oxidation in insects makes it strongly active. It is principally used for the control of sap-sucking insects and mites. It is slightly soluble in water and is of short to moderate persistence. Malathion is susceptible to decomposition by alkalis. It is one of the most generally useful quasi-systemic compounds.

To evaluate the effects of these pesticides on soil microbial activities, three biochemical processes, the dehydrogenase and phosphatase activities in soil and nitrification of added ammonium in soils were chosen.

The enzyme dehydrogenase is involved in the biological oxidation of soil organic matter. This enzyme is very much associated with living microbial cells and it is therefore suitable for measuring effects on

microbial activity.

The phosphatase enzymes are involved in mineralization of organic phosphorus in soil. Soil phosphatase activity has components due to living cells and soil enzymes. Therefore, its activity is less directly dependent upon living cells and less influenced by the effects on the microbial population.

The procedures for measuring activities of both phosphatase and dehydrogenase enzyme systems in soil are simple and need only a simple spectrophotometer. This type of method is suitable for developing countries like Bangladesh.

Nitrification is a more specific process of the oxidation of ammonium to nitrite and nitrite to nitrate by specific microorganisms. Oxidation of NH_4^+ to NO_2^- by Nitrosomonas and oxidation NO_2^- to NO_3^- by Nitrobacter. Any effect on these organisms would be reflected in changes of nitrification rate. So this process is an indicator of microbial activity in soil.

Determination of nitrification rate involves measurement of ammonium, nitrite and nitrate which was done by using a Technicon AutoAnalyzer II system in this Department which is not appropriate for Bangladesh condition. Therefore the determination of inorganic nitrogen by the method of steam distillation based on that of Bremner, 1965, and Keeney and Nelson, 1982, was investigated as this would be more suitable for future

research work under Bangladesh conditions.

The thesis is split up into two parts. The first part is devoted to a comparison of the two methods used for the determination of ammonium, nitrite and nitrate nitrogen, the Technicon automated method and the steam distillation method.

The second part of the thesis is devoted to investigating the effects of the three pesticides on the three selected microbial processes, the dehydrogenase, phosphatase and nitrification in soil.

CHAPTER TWO

METHOD AND MATERIALS

2.1 SOIL SAMPLING SITES

A brief description of the soil sampling sites is given below. Soils were attributed to Soil Series using the Soil Memoirs and soil maps for each area (Grant et al., 1962; Ragg et al., 1976).

Darvel Series

The site is located at Lennoxtown, Scotland. Grid reference No. is NS 635773. The soil is cultivated as a garden. It belongs to the Darvel Association which is formed from fluvioglacial sands and gravels derived from carboniferous igneous and sedimentary rocks. The soil comes under the Darvel Series which has been classed as a freely drained brown forest soil of low base status.

Dreghorn Series

The site is situated at Troon, Ayrshire, Scotland. Grid reference No. is NS 328329. The soil is cultivated as a garden. It belongs to the Dreghorn Association which is developed from raised beach deposits. The Series is Dreghorn which has been classed as freely drained brown forest soil.

Two soils, Darvel and Dreghorn series, were chosen for analysis due to their differing texture and organic matter levels. They had also not been treated with pesticides for at least five years.

2.2 COLLECTION AND PREPARATION OF SOIL SAMPLES

Both the soil samples were taken in the fresh condition from the upper 0-15 cm depth of the soil profile and were brought to the laboratory in labelled plastic bags as soon as possible. The samples were spread on clean plastic sheets and mixed thoroughly to minimise the effects of local variations and partially air dried sufficiently to pass through a sieve with 4 mm opening. Each soil was sieved to remove the larger inert material which is considered to have little effect on the chemical and nutritional status of the soil. A 4 mm sieve ensures as little disruption to the soil and microorganisms as is possible. The microorganisms could die in this stage and also more substrate could be made available to the microbes present due to disruption of the peds. The samples were then stored at 2°C if not used at once.

2.3 DETERMINATION OF SOIL pH

Soil pH was determined in a 5:1 water:soil mixture by a combined glass-reference electrode and pH meter. The meter was first standardized with buffer solutions of

pH 7.0 and pH 4.0. The buffer solutions were prepared by dissolving buffer tablets in 100 ml of deionized water.

Triplicate 4 g samples of each soil were weighed into 2 oz glass bottles and 20 ml deionized water was added to each bottle. The suspension was then shaken for 30 minutes on an end-over-end shaker. The electrode was then immersed in the glass bottle and the soil suspension stirred by swirling the electrode slightly. The pH was read immediately.

The soil pH was also determined exactly the same way by substituting 0.01M calcium chloride solution from deionized water.

2.4 DETERMINATION OF MOISTURE CONTENT

Porcelain basins were washed, and dried in the oven at 110°C for one hour, cooled in a desiccator and the weight recorded. A suitable weight of fresh soil was placed in each basin and weighed. Then the basin containing fresh soil was placed in an oven for 24 hours at 110°C, cooled in a desiccator and reweighed. The % moisture content was determined on an oven dry basis.

$$\%\ \text{moisture} = \frac{\text{weight of water}}{\text{weight of oven dry soil}} \times 100$$

2.5 DETERMINATION OF MOISTURE CONTENT AT -0.5 BAR

Determination of moisture content at -0.5 bar soil moisture potential was carried out with the pressure

plate apparatus. The soil samples were placed on the plate and flooded with water. They were allowed to soak overnight. Excess water was removed from the plate which was then placed in the pressure plate apparatus and the pressure adjusted to 0.5 bar using nitrogen gas from a cylinder. Samples were then allowed to equilibrate for three days, by which time water loss had ceased. The % moisture content was determined on an oven dry basis.

2.6 SOIL PROPERTIES

Some selected properties of soils used in the experiment are given in Tables 2.1 and 2.2.

Soils	LOI%	pH water	pH $CaCl_2$	% moisture at -0.5 bar
Darvel	8.82	6.4	5.4	32.50
Dreghorn	5.64	6.8	5.8	16.52

Table 2.1 Properties of soils

Soils	% Coarse and medium sand*	% Fine sand	% Silt	% Clay	Textural class
Darvel	33.5	20.0	22.0	24.5	Sandy Clay Loam
Dreghorn	86.0	1.9	10.9	1.2	Sand

Table 2.2 Textured properties of soils (Khan, 1987)

* Sand > 180 μm

2.7 METHOD OF PESTICIDE APPLICATION TO SOILS

The pesticides used were unformulated pure chemicals. Captofol was obtained as a high purity standard material of 97% purity from Greyhound Chromatography and Allied Chemicals. Carbaryl was obtained as a certified reference material of greater than 99% purity from the National Physical Laboratory. Technical grade malathion of 89.4% purity was obtained from Cyanamid Great Britain. Captafol and carbaryl were white powders while malathion was in liquid form.

The pesticides were applied at two levels - a low level of 10 mg/kg and 10 times that value. As the required amount of pesticide for the selected levels was not high enough to weigh easily, it was applied at 100 times the low application level (250 mg per 250 g soil) directly onto the soil. This soil was then shaken on a reciprocating shaker at 2°C for two hours to mix thoroughly. A stepwise dilution of the treated soil, using fresh soil, was then carried out to get the maximum field application rate of 10 mg/kg and 10 times that value. 25 g of the treated soil plus 225 g of fresh soil were mixed in the same way to give a final concentration of 25 mg pesticide per 250 g soil for the high level. 25 g of this high level sample plus 225 g fresh soil were mixed to give 2.5 mg pesticide per 250 g of soil for the

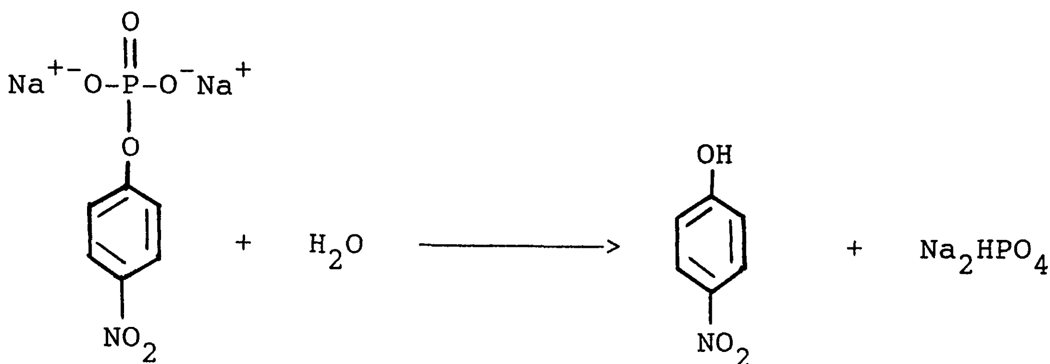
low application level.

This method of application of the pesticides was selected because it avoided the use of a solvent which although it might have given a better distribution of the pesticides in the soil might itself have affected the soil microorganisms and the processes under investigation.

That a satisfactory degree of mixing of the pesticides in the soil was achieved by this method was indicated by the good precision obtained in the subsequent experiments to investigate the effects of the pesticide additions on soil phosphatase and dehydogenase activities (see Section 4.3.1) and nitrification rate (see Section 4.3.2).

2.8 METHOD FOR PHOSPHATASE ACTIVITY

The method used was based on the experiments by Tabatabai and Bremner (1969). This experiment gives an indication of the numbers of soil microorganisms that are capable of cleaving the substrate, disodium p-nitrophenyl phosphate.



The substrate was buffered at pH 6.5 as this was the optimum pH for the phosphatase enzyme. The method used involves colorimetric determination of the p-nitrophenol released after cleavage from the substrate after the soil has been incubated.

NaOH was added after incubation to stop phosphatase activity and extract the nitrophenol produced. This method gives quantitative recovery of p-nitrophenol from the soil. Under alkaline conditions p-nitrophenol gives a yellow colour which is used to measure the amount of nitrophenol present. It was necessary to add $CaCl_2$ to prevent dispersion of the clay which could complicate filtration and the extraction of dark coloured organic matter which would interfere with the colorimetric analysis of p-nitrophenol. Best results are obtained if $CaCl_2$ is added before NaOH.

Toluene is used for two purposes, firstly to stop proliferation of cells and secondly to lyse the cells releasing the phosphatase enzymes for the extracellular reaction.

2.8.1 REAGENTS:

1. Tolune - analar.

2. Modified universal buffer (MUB), pH 6.5:

1.21 g of tris (hydroxymethyl) aminomethane (THAM), 1.16 g of maleic acid, 1.4 g of citric acid, 0.63 g of Boric acid and 1.95 g of sodium hydroxide were taken in a beaker containing 400 ml of water to dissolve and then a magnetic stirring bar was placed in the beaker and the beaker was placed on a magnetic stirrer. The solution was titrated to pH 6.5 with 0.1M HCl and transferred to 500 ml volumetric flask and the volume made up to the mark with water.

3. p-Nitrophenyl phosphate solution, 0.025M:

0.464 g of disodium p-nitrophenyl phosphate hexahydrate was dissolved in about 40 ml of MUB pH 6.5 (for assay of acid phosphatase) in a 50 ml flask and diluted to 50 ml with MUB of the same pH. The solution was stored in a refrigerator.

4. Calcium chloride ($CaCl_2$), 0.5M:

18.375 g of $CaCl_2.2H_2O$ was dissolved in about 175 ml of water and diluted to 250 ml with water.

5. Sodium hydroxide (NaOH), 0.5M:

10 g of NaOH was dissolved in about 350 ml of water

in a volumetric flask and diluted to 500 ml with water.

6. Hydrochloric acid, 0.1M:

10 ml of commercially prepared 1M HCl was taken in a 100 ml volumetric flask and the volume made up to 100 ml using water.

7. Standard p-nitrophenol stock solution (1000 mg/l):

0.1 g of p-nitrophenol was dissolved in about 70 ml water in a volumetric flask and diluted to 100 ml with water and stored in a refrigerator.

Working standards were prepared by dilution in the appropriate extracting solutions.

2.8.2 <u>PROCEDURE</u>

1.00 g of soil was weighed accurately into a universal vial and 0.2 ml of toluene added, followed by 4 ml of MUB pH 6.5 and 1 ml of p-nitrophenyl phosphate solution. The vials were then capped and placed in a shaker inside an incubator and incubated for 1 hour at 37°C. After 1 hour the lids were removed and 1 ml of 0.5M $CaCl_2$ was added followed immediately by 4 ml of 0.5M NaOH. The vials were shaken well and the contents filtered through a Whatman No. 1 folded filter paper. The yellow colour produced was then measured at a wavelength of 420 nm using the Baush Lomb Spectronic 20

Spectrophotometer after a 1 in 10 dilution with water.

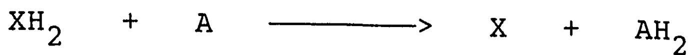
The absorbance reading was then compared to the calibration standard graph for p-nitrophenol prepared using 0, 10, 20, 30, 40 and 50 μg of p-nitrophenol.

The standard graph was prepared by diluting 1 ml of the standard p-nitrophenol solution into a 100 ml flask by making the volume up to 100 ml with MUB of pH 6.5. Then 0, 1, 2, 3, 4 and 5 ml of this solution was pipetted into universal vials and the volume was adjusted to 5 ml with MUB of the same pH. To avoid precipitation of $Ca(OH)_2$ 5 ml of NaOH but no $CaCl_2$ was added. The flasks were shaken and filtered as before. The standard graph is not reproducible and therefore must be prepared on each occasion.

Controls were also performed to ensure that the yellow colour was derived from the p-nitrophenol released by the phosphatase enzyme. To perform controls, the same procedure was followed as described for an assay of phosphatase activity, but in this case no substrate was added prior to incubation. After incubation substrate was added immediately after the addition of $CaCl_2$ and NaOH.

2.9 THE METHOD FOR DEHYDROGENASE ACTIVITY

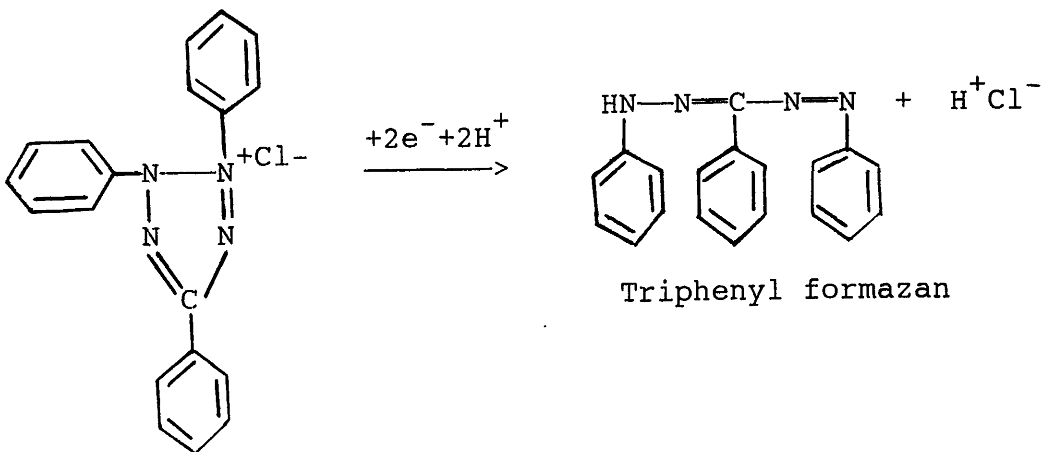
Dehydrogenases are responsible for the biological oxidation of organic compounds. The overall process may be represented as follows:



$$XH_2 + A \longrightarrow X + AH_2$$

where XH_2 is an organic compound and A is a hydrogen acceptor. Many different specific dehydrogenase systems are involved in the dehydrogenase activity of soils. Therefore, the result of the assay of dehydrogenase activity in soil would show the average activity of the active population.

The method used for this experiment was based on the procedure mentioned by Tabatabai (1982). A tetrazolium salt, 2,3,5-triphenyltetrazolium chloride (TTC) is used as substrate. It is a quarternary ammonium salt and as such possesses a high degree of water solubility allowing the experiment to be run in a water solution. TTC is a colourless salt possessing the property of being easily transformed into an intense red, water insoluble, methanol soluble formazan by reduction. This red colour, the triphenyl formazan can be used as a measure of the dehydrogenase activity.



2.9.1 REAGENTS:

1. Calcium carbonate ($CaCO_3$), reagent grade.

2. 2,3,5-Triphenyltetrazolium chloride (TTC), 3%:

3 g of TTC was dissolved in about 80 ml of water and the volume made up to 100 ml with water.

3. Methanol - analar.

4. Triphenyl formazan (TPF) standard solution:

100 mg of TPF was dissolved in about 80 ml of methanol and the volume adjusted to 100 ml with methanol.

2.9.2 PROCEDURE:

6 g of each air dried soil sample and 0.1 g of $CaCO_3$ were placed in 50 ml vial and mixed thoroughly. 1 ml of the 3% aqueous solution of TTC was then added to each vial followed by 2.5 ml of water. The contents of each vial were mixed thoroughly with a glass rod. The vials were then capped and incubated at 37°C for 24 hours. After 24 hours the stoppers were removed and 10 ml of methanol added. Each vial was then shaken for one minute and the suspension was filtered through a glass funnel plugged with absorbent cotton wool, into a 100 ml volumetric flask. Each vial was washed with methanol and the soil quantitatively transferred to the funnel. Then

additional methanol in 10 ml portions was added to the funnel until the reddish colour disappeared from the cotton wool plug. After filtration had been completed the flasks were made up to 100 ml using methanol. The intensity of the reddish colour was then measured by using a Spectronic 20 spectrophotometer at a wavelength of 485 nm using methanol as blank. The amount of TPF produced was calculated by reference to a calibration graph prepared from TPF standards.

The standard graph was prepared by diluting 10 ml of the standard TPF solution in 100 ml of methanol and pipetting 5, 10, 15 and 20 ml into 100 ml flasks and adjusting the volume to 100 ml using methanol. The intensity of the red colour of TPF was measured as described for the samples and the absorbance readings were plotted against the amount of TPF in the 100 ml standard solutions.

2.10 INCUBATION PROCEDURE FOR MEASUREMENT OF NITRIFICATION OF ADDED AMMONIUM

A sample of fresh soil equivalent to 50 g (oven dry weight basis) was weighed into an 8 oz bottle which was left open to permit aeration. The sample was treated with 1 ml of ammonium sulphate solution containing 5,000 mg NH_4-N/litre. Each sample was mixed thoroughly using a glass rod and the moisture content was adjusted to the -0.5 bar moisture potential by the addition of an

appropriate weight of deionized water with a Pasteur pipette. The bottle containing the sample was allowed to stand in the cold room at 2°C for 3 hours. After taking a sample for measuring extractable -N at 0 days' incubation, each 8 oz bottle was then placed inside a large plastic tub which had been lined with dampened filter paper and contained a layer of water on the base to ensure a humid atmosphere in order to keep the samples at the correct moisture content. The plastic tubs were large enough to ensure that there was sufficient oxygen for the incubation period. The lids of the tubs had to be tightly sealed to make it air tight. The tubs were then placed in an incubator set at 25°C. The changes in ammonium, nitrate and nitrite nitrogen were measured at intervals. The plastic tubs were allowed to stand open for 10-15 minutes to replenish the air at the end of each interval. Care was taken to readjust any loss in weight of the soil in the bottle with deionized water before taking a sample for measuring the extractable mineral nitrogen. The ammonium, nitrite and nitrate forms of nitrogen were measured by the method described in Sections 2.11 and 2.12.

2.11 EXTRACTION OF INORGANIC NITROGEN FROM SOIL

The inorganic nitrogen in soil (ammonium, nitrate and nitrite) can be readily determined once it has been brought into solution, but it is necessary that the ions

are brought quantitatively into the extracting solution and that subsequent changes in the concentration with time are prevented.

The nitrate and nitrite nitrogen ions are not held by the soil colloids but are readily and completely extractable when the soil is shaken with water or aqueous solution. When ammonium, nitrate and nitrite all are to be determined on the sample, the extraction procedure needs to be modified because of the presence of ammonium absorbed on the colloidal complex of soil. Most methods use a salt solution such as calcium sulphate, sodium sulphate, potassium sulphate or potassium chloride with an intent to include exchangeable ammonium.

Bremner and Keeney (1966) developed a method for the extraction of inorganic soil nitrogen and determined this by steam distillation method. Comparison of recent reviews shows (Keeney and Nelson, 1982 and Bremner and Hauck, 1982) that there have been no major advances in the method of extraction for inorganic soil nitrogen analysis and the method recommended by Bremner and Keeney (1966) remains the method of choice for most research on nitrogen transformations in soil.

The Soil Science Society of America (1979) has defined exchangeable ammonium as that ammonium which is extractable by neutral potassium salt solution (e.g. 0.5M potassium sulphate, 2M potassium chloride) at room temperature.

Potassium sulphate salt solution was selected as an extractant in this experiment because the extract was satisfactory for use in the automated method of analysis of nitrate and nitrite as well as ammonium used in this department while potassium chloride solution interfered in the automated analysis of nitrate. Flowers and Arnold (1983) also used 0.5M potassium sulphate during inorganic nitrogen analysis of soil and they did not mention any significant interference of the sulphate ion. The soil solution ratio was maintained by using 2.5 g of soil per 50 ml of 0.5M potassium sulphate solution which was compatible with the recommendations of Bremner and Keeney (1966).

2.11.1 WASHING OF GLASSWARE

The shaking bottles, bottle tops, filter funnels, beakers, stirring rods and volumetric flasks were first cleaned with hot water and soaked overnight in a 2% solution of Decon 90 (Decon Laboratories Limited). They were then thoroughly washed with hot water rinsed twice with deionized water and finally dried in a 70°C oven.

2.11.2 WASHING OF FILTER PAPERS

0.5M sulphuric acid was prepared from analar grade concentrated acid using nitrogen-free deionized water. Each filter paper was folded separately into a clean and dry plastic funnel. 50 ml of 0.5M sulphuric acid was

filtered through each filter paper in two equal portions of 25 ml each. The acid washed filter papers were then rinsed 5 times with deionized water to wash away any acid left in the filter paper. Care was taken to make sure that the filter papers were made acid-free. The washed filter papers along with funnels were dried for 4 hours in a 70°C oven before using for filtration (Shah, 1988).

2.11.3 PREPARATION OF 0.5M POTASSIUM SULPHATE SOLUTION

87.125 g potassium sulphate was dissolved in about 800 ml of deionized water and made up to 1 litre. The solution was purified of ammonium nitrogen contamination by raising its pH to 11.00 with 1M potassium hydroxide. It was then boiled and stirred for 15 minutes to give off ammonia gas. The solution was allowed to cool and the pH was readjusted to pH 6.0 with 0.5M H_2SO_4. Deionized water was added for any loss of water during boiling due to evaporation (Khan, 1987).

2.11.4 PROCEDURE FOR EXTRACTION OF INORGANIC-N

An amount of fresh soil equivalent to 2.5 g on an oven dry basis was weighed into a 4 oz glass bottle and was shaken for 2 hours at 2°C with 50 ml of 0.5M potassium sulphate. The suspensions were filtered through previously washed Whatman filter paper No. 1. The first 2 to 3 ml of the filtrates including blanks were discarded. The filtrates obtained were analysed for

ammonium, nitrate and nitrite nitrogen with a Technicon Auto Analyzer II using methods as described in Section 2.12. All the analytical standards used contained equal concentrations of 0.5M potassium sulphate solution as the soil extracts.

2.12 AUTOMATED DETERMINATION OF SOIL INORGANIC NITROGEN

The Technicon AutoAnalyzer II was used in this study for the analysis of ammonium nitrate and nitrite nitrogen, because of its sensitivity, speed and ease of use. The system comprised a sampler, pump, a water bath with constant temperature and a spectrophotometer. Results of the samples were recorded with a single pen chart recorder. The system was connected to a BBC microcomputer which was used for the measurement of peak heights and calculation of results.

2.12.1 DETERMINATION OF AMMONIUM NITROGEN

Ammonium-N was determined by a modification of the indophenol green method using a complexing reagent to prevent interferences due to the precipitation of hyroxides in the reagent system. With the inclusion of a sodium nitroprusside catalyst the sensitivity of the method was such that ammonium could be determined in the range of 0 to 1 ppm and with care 0 to 0.1 ppm (Brown, 1973). The schematic diagram of the flow system is shown in Figure 2.1.

2.12.1.1 REAGENTS

Analar grade reagents and nitrogen free deionized water were used throughout.

1. Alkaline phenol.

22.5 g of sodium hydroxide was dissolved in about 900 ml deionized water in a 1 litre dark glass bottle and the resulting solution was degassed. 50 g phenol was taken in a 1 litre beaker and approximately 500 ml sodium hydroxide solution was added and stirred carefully with a glass rod to dissolve the phenol. The solution was returned to the bottle and the volume made to 1 litre with degassed water and mixed gently.

2. Complexing reagent.

50 g potassium sodium tartrate and 50 g sodium citrate were dissolved in approximately 900 ml deionized water in a 1 litre bottle and degassed. 1.2 g sodium nitroprusside was taken in a 100 ml beaker. 50 ml degassed water was added to the beaker and stirred gently with a magnetic stirrer. The resulting solution was added to the citrate-tartrate solution. 0.5 ml of 30% Brij-35 was added and the volume made to 1 litre. The solution was then mixed gently.

3. Sodium hypochlorite solution (0.5%).

50 cm^3 sodium hypochlorite solution (12% w/v available chlorine) was diluted to 1 litre with degassed deionized water and mixed gently.

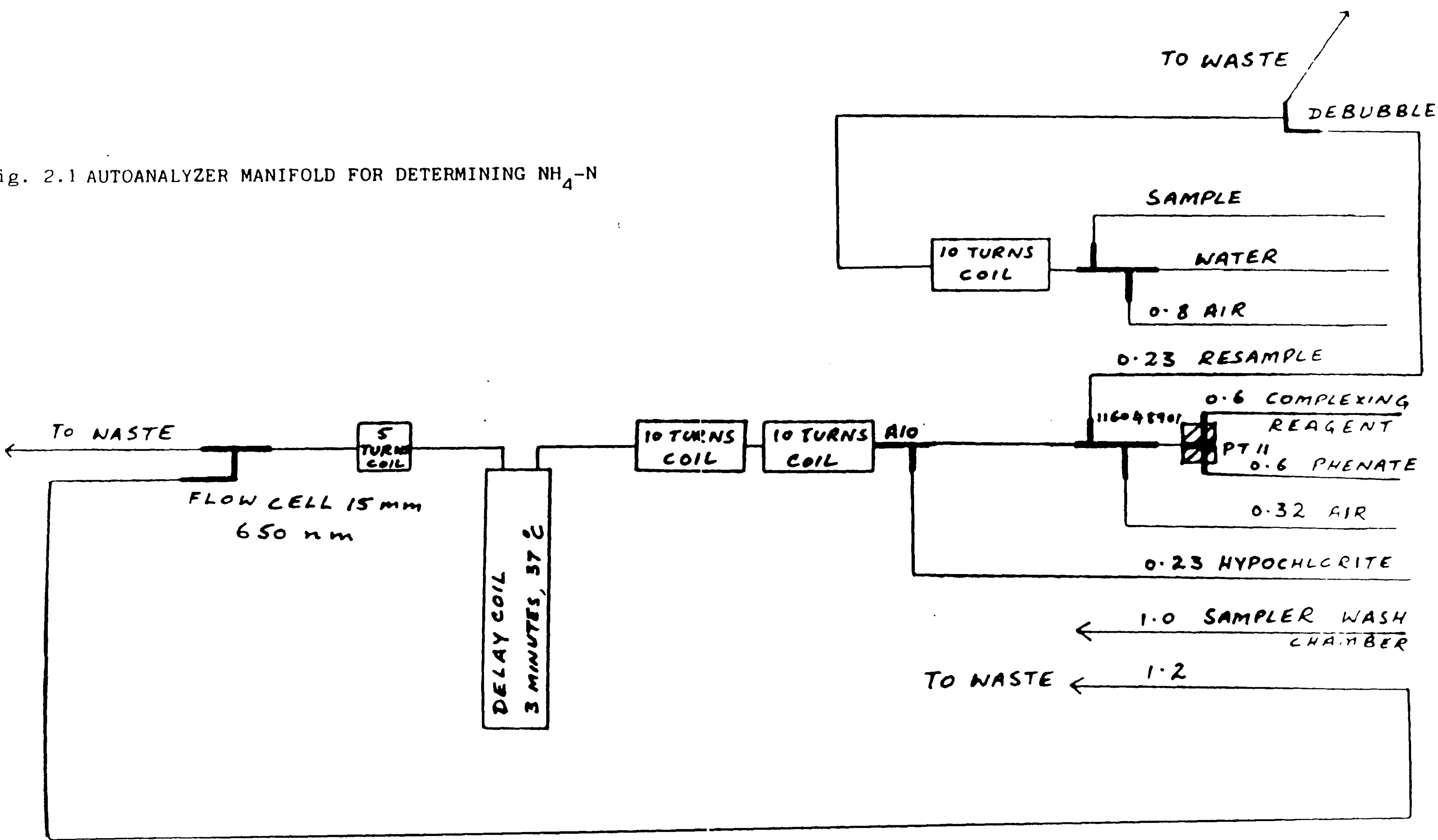
4. Ammonium-N standard stock solution (1000 mg/l).

Ammonium sulphate was dried for one hour at 110°C and cooled in a dessicator. 4.718 g dried ammonium sulphate was dissolved in deionizing water and the volume made up to 1 litre. This stock solution was stored at 2°C. Working standards were prepared by dilution in the appropriate extracting solutions.

2.12.1.2 PROCEDURE

The filtered solutions were analysed using the Technicon AutoAnalyser II including standard solutions, blanks and zeros. The samples were run at the rate of 50 per hour and the colour development was carried out in the water bath at 37°C. The colour intensity was measured at 650 nm. As the calibration graph for ammonium is linear, only two standards were required - 5 ppm and 0 ppm. Samples with ammonium-N concentrations above 5 ppm were diluted into the range 0 to 5 ppm using an inbuilt dilution step.

Fig. 2.1 AUTOANALYZER MANIFOLD FOR DETERMINING NH_4-N



2.12.2 NITRATE AND NITRITE NITROGEN DETERMINATION

In the automated system, nitrate nitrogen was quantitatively reduced to nitrite nitrogen followed by determination of the nitrite using the Greiss reagent. The method, therefore, measured nitrate plus nitrite. The nitrite nitrogen was measured separately on the same manifold by omitting the reduction reagents.

The schematic diagram of the flow system for nitrate and nitrite is shown in Figure 2.2 (Best, 1976).

2.12.2.1 REAGENTS

Analar grade reagents and nitrogen free deionized water were used throughout.

1. Buffer solution.

22.5 g sodium tetraborate and 2.5 g sodium hydroxide were dissolved in about 900 ml deionized water and the volume made to 1 litre. The solution was then degassed.

2. Greiss reagent.

100 ml of concentrated hydrochloric acid was added into approximately 800 ml deionized water and the solution was degassed. 10.0 g sulphanilamide and 0.5 g N-1-naphthylene diamine dihydrochloride were taken into a 1 litre beaker. 500 ml of the acid solution was added to the beaker and stirred gently using a magnetic stirrer. This solution was returned to the bottle, and the volume

made to 1 litre with degassed water and mixed gently. The solution was stored at 2°C.

3. Reducing reagent.

The reducing reagent was divided into two components.

a) Hydrazine sulphate.

0.30 g of hydrazine sulphate was taken in a small beaker and transferred carefully to a 1 litre volumetric flask containing approximately 900 ml degassed deionized water. The flask was made up to the mark without shaking and hydrazine sulphate was dissolved by stirring with a magnetic stirrer keeping the top of the flask closed in order to prevent access of oxygen. 0.5 ml of 30% Brij-35 solution was then added and mixed gently.

b) Catalyst solution

1 ml of 2.47% copper sulphate solution and 0.5 ml of 30% Brij-35 solution were added to 1 litre of degassed water and mixed gently.

For the determination of nitrite nitrogen the reducing reagents were replaced with nitrogen free deionized water containing 0.5 ml per litre of 30% Brij-35 solution.

4. Nitrate nitrogen standard stock solution (1000 mg/l).

Potassium nitrate was dried for one hour at 110°C and cooled in a dessicator. 7.222 g of dried potasium nitrate was dissolved in deionized water and the volume made to 1 litre. The stock solution was stored at 2°C. Working standards were prepared by dilution in the appropriate extracting solutions.

5. Nitrite nitrogen standard stock solution (1000 mg/l).

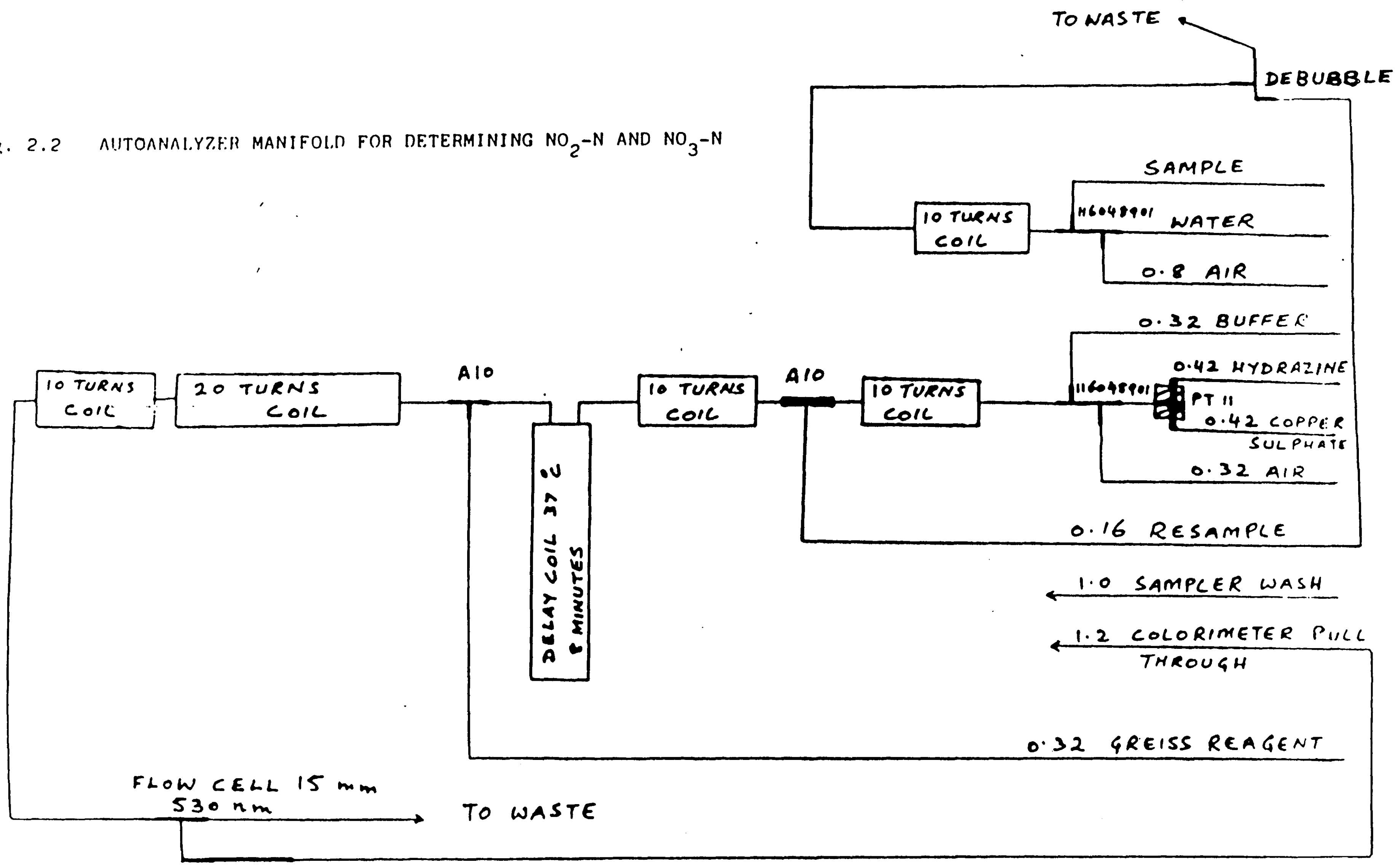
Sodium nitrite was dried for one hour at 110°C and cooled in a dessicator. 4.928 g of dried sodium nitrite was dissolved in deionized water and the volume made to 1 litre. The solution was stored at 2°C. Working standards were prepared by dilution in the appropriate extracting solutions.

2.12.2.2 PROCEDURE:

The extracts were run through the Auto Analyzer together with the appropriate standards, blanks and zeros. The samples were run at the rate of 50 per hour. The nitrate nitrogen was reduced to nitrite by adding copper sulphate and hydrazine sulphate solutions to the sample as it passed through the water bath set at 37°C. The nitrite nitrogen was determined by a diazotization coupling reaction whereby a pink colour was formed. The

intensity of this colour was measured at 530 nm. Nitrate has a curved calibration 0-5 ppm. Nitrite was determined in the same way as nitrate except the reduction step was omitted. Nitrite has a linear calibration in the range of 0-1 ppm. Samples with nitrate-N concentrations above 5 ppm were diluted into the range 0 to 5 ppm using an inbuilt dilution.

Fig. 2.2 AUTOANALYZER MANIFOLD FOR DETERMINING NO_2-N AND NO_3-N



2.13 STEAM DISTILLATION METHODS FOR DETERMINATION OF INORGANIC-N

The steam distillation methods used for the determination of inorganic-N developed from the finding that interferences by glucosamine and other alkali-labile organic-N compounds in alkali distillation methods of determining NH_4^+ are eliminated if the distillation is performed with steam using a small amount of MgO and a short period of distillation. The methods are simple, rapid and are not affected by various organic and inorganic substances that often interfere with colorimetric methods of determining inorganic-N (Bremner, 1965, Bremner and Keeney, 1965, Keeney and Nelson, 1982).

Ammonium is converted entirely to ammonia at pH greater than 10 and the function of MgO in the distillation is to bring about such a pH. It also eliminates interference from glucosamine and other alkali-labile organic-N compounds. Boric acid solution is used to trap ammonia from the distillate. Devarda alloy is employed for the reduction of NO_3^- and NO_2^- to ammonium permitting the determination of NO_3^- and NO_2^-. Distillates are titrated against 0.005N sulphuric acid to determine the amount of N present.

2.13.1 APPARATUS

Steam distillation apparatus (Figure 2.3):

This apparatus is designed so that round bottomed flasks fitted with standard-taper (19/38) ground glass joints can be used as distillation chambers. The flasks are provided with glass hooks so that they can be fastened to the distillation apparatus by spiral steel springs with loop ends. The steam required for distillation is generated by heating distilled water in a 5-litre flask that contains anti-bump granules and a small amount of sulphuric acid to trap any NH_4^+ in the distilled water. The distillation apparatus should be steamed out for about 10 minutes to remove traces of ammonia before use and the rate of steam generation should be adjusted so that 7 to 8 ml of distillate are collected per minute. The desired rate of distillation is readily obtained by heating the steam generator flask with an electric heating mantle. The flow of cold water through the condenser of the apparatus should be such that the temperature of the distillate obtained using this rate of distillation does not exceed 22°C. The trap at the base of the water jacket on the condenser is to prevent water condensing on the external surface of the condenser from entering the flask used to collect the distillate.

2.13.2 REAGENTS

1. Magnesium oxide (MgO):

MgO was pretreated by heating at 700°C for two hours, cooled in a dessicator and stored in an air tight bottle. Each of these measures was designed to remove CO_2 from the MgO (e.g. $MgCO_3$), which interferes with analysis at the titration stage, particularly in acid extracts.

2. Sulphuric acid (H_2SO_4), 1N volumetric standard solution - BDH.

3. 0.005N sulphuric acid:

The 0.005N H_2SO_4 was prepared by diluting 5 ml of 1N H_2SO_4 in 1000 ml of water in a volumetric flask.

4. Tris hydroxymethyl amino methane (THAM), 0.01N:

The THAM buffer was prepared by dissolving 12.114 g of THAM in 100 ml of water to give 1N THAM and 1 ml of this 1N THAM diluted to 100 ml to give 0.01N THAM.

5. Boric acid indicator mixture:

To 250 ml of 2% boric acid, 2 ml of 0.5% methylene blue in ethanol was added followed by 3 ml of 0.1% methyl red in ethanol and 1 ml of 0.1N NaOH solution. The volume of the methyl red used was found by trial and error, based on the sharpness of the endpoint when the

solution diluted with equal volume of water to give a green colour and titrated against 0.005N sulphuric acid to give a permanent, faint pink colour.

6. NH_4-N standard stock solution (1000 mg/l):

1000 ppm of ammonium stock solution was prepared as described in Section 2.12.1.1.

7. NO_3-N stock solution (1000 mg/l):

1000 ppm of nitrate-N stock solution was prepared by the method as described in Section 2.12.2.1.

8. Working standards:

5 ppm of both ammonium and nitrate-N standards were prepared by diluting 5 ml of the respective 1000 ppm standard stock solution in 1000 ml of water in a volumetric flask.

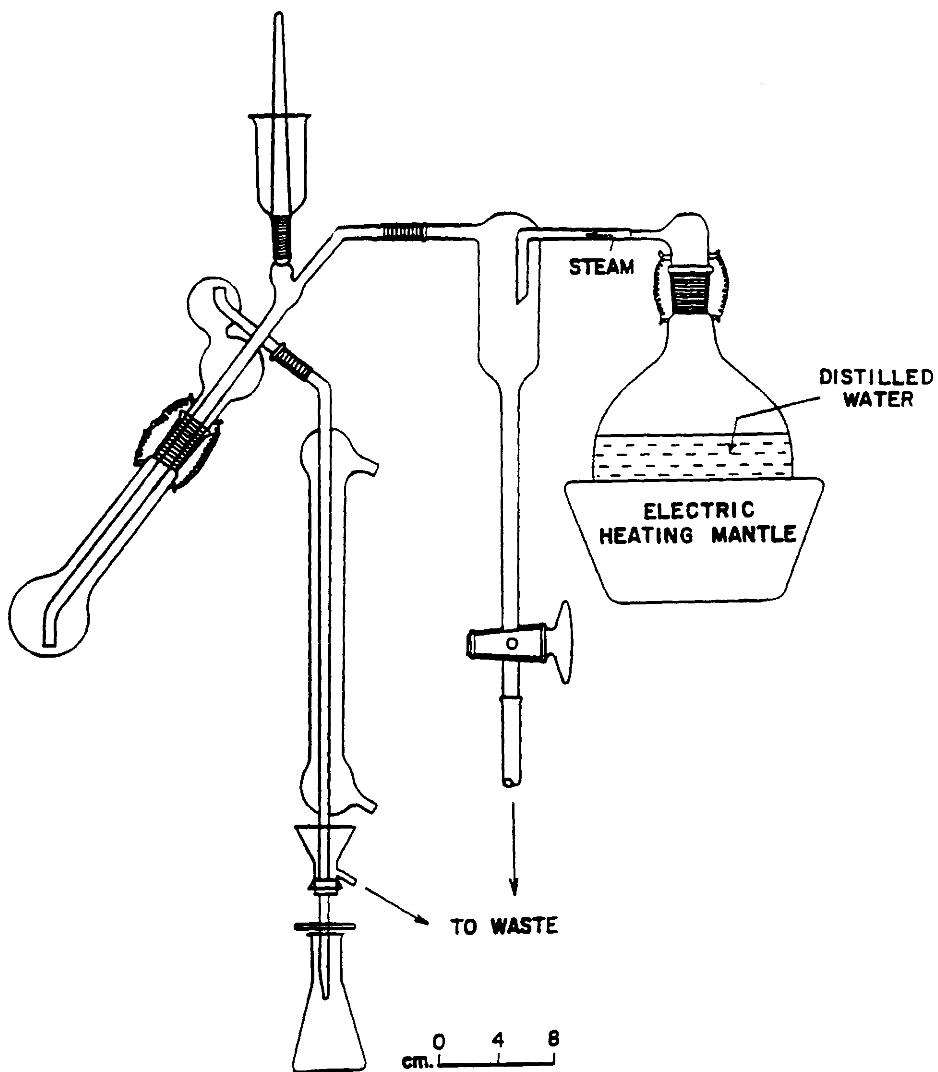


Fig. 2 .3 Steam distillation apparatus.

2.13.3 PROCEDURE

2.13.3.1 STANDARDIZATION OF 0.005N SULPHURIC ACID:

To 5 ml of 0.01N THAM, 20 ml of water was added with few drops of methyl red indicator. The solution was then titrated with 0.005N H_2SO_4. The exact normality of the sulphuric acid can be calculated as

$$N = \frac{(5*0.01)}{\text{titration volume (ml)}}$$

2.13.3.2 DETERMINATION OF NH_4-N:

6 ml of boric acid indicator mixture was taken into a 50 ml flask and the flask placed under the condenser of the steam distillation apparatus so that the end of the condenser was about 1 cm below the surface of the boric acid mixture.

Up to 25 ml of sample was taken into a distillation flask and 0.2 g of MgO added to it. The flask was attached to the steam distillation apparatus as shown in Figure 2.3 and steam distillation immediately commenced by closing the stop cock on the steam bypass tube of the distillation apparatus. When the distillate reached the 20 ml mark on the receiver flask the flask was lowered so that the end of the condenser was about 4 cm above the surface of the boric acid mixture. The distillate when it reached the 35 ml mark on the receiver flask, the distillation was stopped by opening the stopcock on the

steam bypass tube and end of condenser rinsed by the water. The NH_4^+-N was determined by titration with 0.005N H_2SO_4 from a micro burette. The colour change at the endpoint was from green to a permanent, faint pink.

To perform a blank, the same procedure was followed as described for the sample by taking 25 ml deionized water.

2.13.3.3 DETERMINATION OF NO_3-N+NO_2^--N

After removal of NH_4^+-N from the samples as described in the previous section, 0.2 g of Devarda alloy was added to the flask which was then reconnected to the steam distillation apparatus. 6 ml of boric acid was taken in a new flask and distillation repeated for the determination of ammonium, collecting a further 35 ml of distillate. Then the NH_4^+-N liberated by the reduction and steam distillation was determined by titration with 0.005N sulphuric acid.

To perform a blank determination, the same procedure as described for the sample was followed by taking 25 ml of deionized water.

2.13.3.4 DETERMINATION OF NO_3^--N

To determine NO_3^- in the presence of NO_2^- the same procedure was followed as described in the previous section, but the sample in the distillation flask was treated with 1 ml of sulfamic acid and the flask swirled

for a few seconds to destroy NO_2^- before the addition of Devarda alloy. This procedure determines NO_3^- only.

To perform a blank determination the same procedure was followed as described for the sample by taking 25 ml of deionized water.

2.13.3.5 CALCULATION

Concentration of N (μg/ml) in the sample was calculated as follows

1 ml of 0.005N H_2SO_4 = 70 μgN

Wt of N (μg) in sample

= (sample titre - blank titre) * 14 * N acid * 1000

= (sample titre - blank titre) * 70[a]

Concentration of N (μg/ml)

$$= \frac{\text{(sample titre - blank titre) * 70}}{\text{Volume of sample}}$$

a 70 μgN corresponds to 0.005N sulphuric acid.

CHAPTER THREE

A COMPARISON OF THE STEAM DISTILLATION AND TECHNICON AUTOMATED METHODS FOR DETERMINATION OF INORGANIC NITROGEN

3.1 INTRODUCTION

Potentiometric, colorimetric and distillation methods for the determination of inorganic forms of nitrogen (ammonium, nitrate and nitrite nitrogen) are all used in soil and water analytical laboratories. The limitations imposed by the expense, rapidity, versatility and precision of the individual methods are the deciding factors in selecting which methods are to be used in a particular laboratory. A large number of soil inorganic nitrogen extractants like potassium chloride, potassium sulphate, sodium chloride, calcium chloride and potassium acetate are all available and the adoption of a single solution for the extraction of soil is also important for routine inorganic nitrogen determinations.

The distillation methods are simple, accurate and they are not affected by various organic and inorganic substances that often interfere with colorimetric methods of determining inorganic nitrogen. They have further advantages over colorimetric methods in that they are applicable to coloured extracts and permit isotope-ratio analysis of exchangeable NH_4^+-N, NO_3^--N and NO_2^--N in

tracer studies concerning the fate of ^{15}N-enriched compounds in soil.

The determination of inorganic forms of nitrogen by specific ion electrodes would, on first consideration, be quite attractive when compared with manual distillation or colorimetric methods. In theory, this approach should be simpler, more rapid, and less expensive. However, specific ion electrodes are expensive, and the cost of a suitable meter-electrode combination can easily exceed the costs of distillation units or simple spectrophotometers. Further, the NO_3^- electrode, requires continual restandardization, is subject to numerous interferences, and is not as sensitive as distillation or colorimetric procedures.

The distillation procedures are time consuming and the potentiometric methods, particularly the nitrate electrode, are prone to chloride ion interference. The use of automated colorimetric analysis for determination of inorganic nitrogen in various extracting solutions is an attractive alternative because large numbers of samples can be analysed quickly and with a high degree of reproducibility.

In a recent development any AutoAnalyzer system can be upgraded by adding microprocessor based controller and data handler. The sampling rate and sample to wash ratio may be set to any desired rate and ratio. As a data handler, the microprocessor can provide drift, gain and

carry over corrections through peak height adjustments. Also the microprocessor produces a printed record of the calculated and corrected final analytical results.

The aim of the work described in this section is to compare two methods, the steam distillation method and the Technicon automated colorimetric method for the determination of inorganic forms of nitrogen.

In developing countries like Bangladesh steam distillation methods for the determination of inorganic nitrogen are widely used because the steam distillation methods are very simple and less expensive than any other methods. Irregular supply of electricity is a major problem in Bangladesh. Fluctuations in voltage and failing electricity supply can cause problems with meter stability and electrodes in the specific electrode methods. Automated colorimetric methods are very sophisticated and there are technical difficulties in running such complex systems. The repair and maintenance of these complicated instruments are also very difficult. Moreover, these methods require skilled technicians and well furnished laboratories which are lacking in Bangladesh. For these reasons steam distillation methods have received wide acceptance in Bangladesh. In order to become acquainted with modern technology the Technicon AutoAnalyzer system normally used in the Agricultural Food and Environmental Chemistry laboratory was also used in this study.

Both the steam distillation method and Technicon AutoAnalyzer method have a limit of detection. The 5 ml microburette used in the distillation method was graduated at 0.02 ml intervals. There are a blank and a sample titration, giving an overall error of approximately 0.04 ml. For a 25 ml sample this would correspond to an error in concentration of 0.11 μg/ml. Using the Technicon AutoAnalyzer an amount as low as 0.001 μg/ml can be detected. Levels of detection in soil depend on the ratio between weight of soil and volume of extractant. In steam distillation methods, for the analysis of soil extracts, different extractants like 4 M KCl at a solution to soil ratio of 5:1 or 2 M KCl at a solution to soil ratio of 10:1 can be used to produce a relatively concentrated extract. For the Technicon automated method used in this department, 0.5 M K_2SO_4 is used as the extractant at a solution to soil ratio of 20:1. This is necessary because of the interference by chloride in the colorimetric determination of NO_3^- and the low solubility of K_2SO_4.

3.2 METHODS

To test the precision and accuracy of the steam distillation and Technicon methods, accuracy being defined by the % recovery of a standard solution, a 5 ppm standard of both ammonium and nitrate nitrogen was used for steam distillation method and a 1 ppm standard of

both ammonium and nitrate nitrogen was used for the Technicon.

3.2.1 STEAM DISTILLATION METHODS

Ammonium-N

25.0 ml samples of 5 ppm ammonium-N standard were distilled in 10 replicates, as were blanks of 25 ml deionized water. Immediately prior to distillation 0.2 g MgO was added to the distillation flask, which was then connected to the apparatus. The procedure for the determination of NH_4-N was followed as described in Section 2.13.3.2.

Nitrate-N

25.0 ml samples of 5 ppm nitrate-N standard were distilled in 10 replicates, as were blanks of 25 ml deionized water. After the addition of 0.2 g MgO, 0.2 g Devarda alloy was added to the distillation flask which was then connected to the apparatus. The amount of N was determined by following the procedure as described in Section 2.13.3.4. In this experiment there was no need for a distillation in absence of Devarda alloy to remove NH_4-N.

3.2.2 TECHNICON METHOD

Colorimetric analysis of ammonium and nitrate nitrogen was carried out on a Technicon AutoAnalyzer II

in conjunction with a chart recorder and a BBC microcomputer.

Cleaning of volumetric flasks: All the volumetric flasks used in the experiments were first cleaned with hot water and soaked overnight in a 2% solution of Decon 90. They were then thoroughly washed with hot water, rinsed twice with deionized water and finally dried in a 70°C oven.

Experiment 1

Ten volumetric flasks were chosen and labelled so that they retained their numbers throughout the experiment. 10 replicate 1 mg/l ammonium-N standard solution in water were prepared in 10 clean 100 ml volumetric flasks. They were analysed by Technicon AutoAnalyzer II using appropriate blanks and zeros. The experiment was repeated three times with newly prepared standard solutions and using the same flasks, which were cleaned thoroughly before use each time.

Experiment 2

Three 1 mg/l ammonium-N standard solutions in water were prepared in three clean 100 ml volumetric flasks. 10 replicate subsamples from each flask were then analysed.

Experiment 3

A volumetric flasks (Number 9) which gave unexpectedly low results in experiment 1 was replaced and experiment 1 repeated two further times.

Experiment 4

10 volumetric flasks were selected and labelled so that they retained their numbers throughout the experiment, 10 replicate 1 mg/l nitrate-N standard solutions in water were prepared in 10 clean 100 ml volumetric flasks. They were analysed by Technicon AutoAnalyzer II using appropriate blanks and zeros. The experiment was repeated twice with newly prepared solutions using the same flasks, which were cleaned thoroughly before use each time.

Experiment 5

Two 1 mg/l nitrate-N standard solutions in water were prepared in two cleaned 100 ml volumetric flasks. 10 replicate subsamples from each flask were then analysed.

Experiment 6

In this experiment the effect of cleaning the volumetric flasks by different methods was investigated. 20 volumetric flasks were chosen and labelled so that they retained their numbers throughout the experiment.

The flasks were cleaned by soaking in Decon 90 as described previously. Twenty 1 mg/l ammonium-N standard solutions were prepared and analysed. This experiment was repeated twice more using the same flasks.

The 20 flasks were then cleaned by soaking overnight in chromic acid and thoroughly washed with hot water, rinsed twice with deionized water. 1 mg/l ammonium-N standard solutions were prepared and analysed. Finally the flasks were cleaned by soaking in Decon 90 as described before and 1 mg/l ammonium-N standard solutions were prepared in water and analysed.

Experiment 7

20 replicate 1 mg/l ammonium-N standard solutions were prepared in 2 M KCl and analysed by Technicon AutoAnalyzer II. These were compared with 1 mg/l ammonium-N standard solutions prepared in water.

Experiment 8

20 replicate 1 mg/l nitrate-N standard solutions prepared in water and 2 M KCl were analysed by Technicon AutoAnalyzer II using appropriate blanks and zeros.

3.3 RESULTS AND DISCUSSION

By the steam distillation method the % recovery achieved for the NH_4-N standard was 95.2% (Table 3.1) and in the case of nitrate nitrogen 94% (Table 3.2). From the low percent recovery, it seems that the major factor affecting recovery of nitrogen in the steam distillation system used was the loss of ammonia at the joint between the spray trap and the distillation flask.

To improve the percent recovery and to prevent the loss of ammonia, the joint on the apparatus was modified. Tests achieved recoveries of NH_4-N of 99.6% and NO_3-N 99.6% (Liken, Personal communication).

Analysis of ammonium-N standard solution prepared in 10 different volumetric flasks by the Technicon AutoAnalyzer II on three occasions gave means of 1.015, 1.005 and 1.015 and standard deviations of 0.0275, 0.0215 and 0.0225 (Table 3.3). For the replicate analysis of solution from a single flask the means were 1.022, 1.010 and 1.019 and standard deviations of 0.0091, 0.0040 and 0.0018 (Table 3.4).

Sample titration volume	Average blank volume	μg/ml N	Mean	Standard deviation	% recovery
2.28	0.596	4.76	4.76	0.0193	95.2
2.28		4.76			
2.28		4.76			
2.28		4.76			
2.26		4.71			
2.28		4.76			
2.29		4.79			
2.28		4.76			
2.28		4.76			
2.28		4.76			

Table 3.1 Analysis of ammonium-N by steam distillation method.

Sample titration volume	Average blank volume	μg N/ ml	Mean	Standard deviation	% recovery
2.12	0.454	4.71	4.70	0.0242	94
2.12		4.71			
2.12		4.71			
2.12		4.71			
2.12		4.71			
2.10		4.66			
2.10		4.66			
2.10		4.66			
2.12		4.71			
		4.71			

Table 3.2 Analysis of nitrate-N by steam distillation method.

Flask Number	Run Number		
	1	2	3
	------------ mg N/litre ------------		
1	0.993	0.994	1.000
2	1.030	1.015	1.033
3	1.013	1.005	1.013
4	1.029	1.008	1.026
5	1.028	1.015	1.019
6	1.021	1.010	1.021
7	1.038	1.023	1.033
8	1.020	1.013	1.013
9	0.946	0.948	0.959
10	1.033	1.018	1.030
Mean	1.015	1.005	1.015
Standard deviation	0.0275	0.0215	0.0225

Table 3.3 Analysis of 1 ppm solution on NH_4-N prepared in 10 different volumetric flasks on three occasions.

Replicate Number	Flask		
	A	B	C
	------------ mg N/litre ------------		
1	1.006	1.005	1.018
2	1.012	1.011	1.020
3	1.025	1.015	1.018
4	1.016	1.016	1.017
5	1.019	1.008	1.023
6	1.022	1.006	1.019
7	1.019	1.011	1.017
8	1.034	1.007	1.019
9	1.031	1.005	1.018
10	1.032	1.011	1.017
Mean	1.022	1.010	1.019
Standard deviation	0.0091	0.0040	0.0018

Table 3.4 Repeated analysis (10 replicates) of 1 ppm NH_4-N solution in three volumetric flasks A, B and C.

In the preparation of solution variability is mainly associated with the pipette and the volumetric flasks used in the experiment. A 1 ml bulb pipette was used which has a tolerance and random error of ±0.015 ml and ±0.02 ml respectively giving a total variability of ±0.035 ml in 1 ml which is equivalent to 3.51%. The flask has systematic and random errors of ±0.15 ml and ±0.02 ml respectively giving a total error of 0.17 ml in 100 ml which is equivalent to 0.17%. There are also personal variability due to reading the line on pipette and volumetric flasks, the drain time of last drop of pipette, addition of last nearest drop to the mark of the volumetric flask.

The analysis of solution from a single flask the source of variability is associated with the Technicon AutoAnalyzer II which may be derived from the cups contaminated by the fingers of operator, accidental transfer of solution from one cup to another and the other possibility is how the cups are manufactured. Colour reaction may cause variability due to the interference of organic and inorganic substances. If the flow of the system is not smooth that also affects the results. Noise on the trace of chart recorder can cause problems, since in measuring peak height there is a possibility of variation. The Technicon measures the concentration of the samples by comparing with a primary standard. If the standard is wrong that will make

variation. Drift can cause variation though it is corrected partly by the computer program which calculates the result. If the drift is very irregular then correction by computer is poor.

The results of the analysis of 1 mg/l ammonium-N standard solution prepared in 10 different volumetric flasks showed unexpectedly high standard deviations for the variability involved with the preparation of solutions.

Results obtained from the repeated analysis of a single flask on the Technicon AutoAnalyzer II were found to be very reproducible as shown by the low standard deviation. From the unexpectedly high standard deviation of the ten different flasks it seems that the cause of variability was due to the flasks because it was noted that the flask number 9 had a surprisingly lower measured concentration than any other flask all three times the solutions were prepared. Flask No. 1 also gave a somewhat lower concentration consistently. Ignoring flasks No. 9 and 1 the standard deviation was found to be 0.008 which is lower than for all ten flasks.

Two sources of variability were identified, firstly touching rims of cups with fingers and secondly the flasks. Precautions were taken by wearing gloves to avoid the touching of cups and by replacing the flask No. 9 which gave the low concentration. The experiment was repeated with 1 mg/l NH_4-N standard solution prepared in

10 different flasks which gave means of 0.996 and 0.996 and standard deviations of 0.0044 and 0.0049 (Table 3.5). The standard deviations were not much greater than for the repeated analysis of the solution that indicating that the sources of variability in solution preparation had been decreased.

Analysis of 1 mg/l nitrate-N standard solution prepared in 10 different volumetric flasks gave means of 1.000 and 1.000 and standard deviations of 0.0044 and 0.0029 in two occasions (Table 3.6). Analysis of 10 replicates from single flask gave means of 0.999 and 0.999 and standard deviations of 0.0015 and 0.0018 (Table 3.7). These standard deviations are similar to the best achieved NH_4-N determination without the need for any special precautions. So the problems of variability were associated with the preparation of ammonium-N standard.

To test the calibration of the volumetric flasks as a cause of variability in determining the NH_4-N by the Technicon AutoAnalyzer II, all 10 flasks initially used in the experiment were weighed and then filled with water up to the mark and reweighed. The actual weight of water was calculated and then dividing the weight of water by the density of water the volume of the flasks were determined. The mean of the volume 10 different volumetric flasks was found 99.84567 and the standard deviation 0.05465 (Table 3.8). So the variability was not due to an error in the volume of flasks No. 9 and 1.

Flask Number	Run Number	
	1	2
	------- mg N/litre ------	
1	0.996	0.995
2	1.000	1.000
3	0.999	1.001
4	0.995	0.995
5	0.998	0.998
6	1.002	1.001
7	0.986	0.985
8	0.994	0.991
9	0.995	0.997
10	0.995	0.995
Mean	0.996	0.996
Standard deviation	0.0044	0.0049

Table 3.5 Analysis of 1 ppm solution of NH_4-N prepared in 10 different volumetric flasks on two occasions.

Flask Number	Run Number	
	1	2
	------- mg N/litre ------	
1	0.997	0.998
2	0.997	1.002
3	1.005	0.998
4	0.997	0.994
5	0.997	1.000
6	1.000	1.002
7	1.006	1.002
8	0.999	1.004
9	1.005	1.002
10	0.94	1.001
Mean	1.000	1.000
Standard deviation	0.0044	0.0029

Table 3.6 Analysis of 1 ppm solution of NO_3-N prepared in 10 different volumetric flasks on two occasions.

Replicate Number	Flask A	Flask B
	mg N/litre	
1	0.997	0.997
2	1.002	0.997
3	0.998	1.000
4	0.999	1.001
5	0.998	0.997
6	0.999	0.999
7	0.997	0.996
8	0.997	1.001
9	0.999	0.999
10	0.999	0.998
Mean	0.999	0.999
Standard deviation	0.0015	0.0018

Table 3.7 Repeated analysis of 10 replicates 1 ppm NO_3-N solution prepared in two volumetric flasks A and B.

Flask number	Weight of empty flasks	Weight of flask with water	Weight of water only	Volume of the flasks
1	49.6386	149.2210	99.5824	99.85205
2	45.2926	144.8675	99.5749	99.84453
3	51.3822	150.9333	99.5511	99.82067
4	59.7395	159.4400	99.7005	99.97047
5	44.6092	144.1401	99.5309	99.80041
6	58.9943	158.6274	99.6331	99.90289
7	47.3914	146.9161	99.5247	99.78419
8	61.7040	161.2865	99.5824	99.85215
9	53.7648	153.3088	99.5440	99.81355
10	47.8239	147.3602	99.5363	99.80583

Mean 99.84567
Standard deviation 0.05465

Table 3.8 Determination of the calibration of volumetric flasks

In order to check the cleaning of the flasks, 1 mg/l ammonium-N standard solutions were prepared in 20 different volumetric flasks cleaned with Decon 90. Solution prepared on three occasions gave means of 1.018, 1.018 and 1.027 and standard deviations of 0.0137, 0.0126 and 0.0114. Following cleaning of these flasks with chromic acid the standard solution prepared in them gave a mean of 1.017 and a lower standard deviation of 0.0065. After cleaning these flasks with Decon 90 the standard solution prepared gave a mean of 1.025 and an increased standard deviation of 0.0100 (Table 3.9).

From the results obtained from the analysis of ammonium-N it is noted that after soaking the flasks with chromic acid the standard deviation was comparatively lower but again cleaning with Decon 90 gave a higher standard deviation for standards prepared in water.

Further investigations were made using ammonium and nitrate-N standard solutions prepared in water and 2 M KCl (Table 3.10 and 3.11). The standard deviation for 20 ammonium-N standards prepared in water was higher than for ammonium standard prepared in 2 M KCl and nitrate standard prepared in either water or 2 M KCl.

A possible reason for variability in measuring NH_4-N standard solutions in water is due to the adsorption of NH_4^+ ion by negative sites on the volumetric flasks. NO_3^- would not be affected by such negative adsorption sites and in 2 M KCl, K^+ would be adsorbed in preference

to NH_4^+ as K^+ would be present at a much higher concentration.

Variability among different flasks may be due to damage to the glass surface caused by solutions previously stored in the flasks. Cleaning in alkaline solution of Decon 90 may have made the problem worse compared to cleaning in chromic acid.

	Cleaning solutions				
	Decon	Decon	Decon	Chr Acid	Decon
	mg N/litre				
	1.020	1.018	1.036	1.020	1.030
	1.027	1.028	1.035	1.025	1.033
	1.024	1.017	1.034	1.024	1.021
	1.038	1.023	1.034	1.022	1.034
	1.002	0.995	1.009	1.007	1.011
	1.013	1.014	1.028	1.019	1.019
	1.034	1.015	1.029	1.021	1.029
	1.025	1.017	1.024	1.018	1.032
	1.023	1.020	1.034	1.023	1.025
	0.983	0.989	0.991	1.016	1.034
	1.028	1.032	1.023	1.018	1.032
	1.019	1.009	1.018	1.009	1.021
	1.014	1.009	1.039	1.012	1.033
	1.010	1.010	1.029	1.013	1.030
	1.013	1.014	1.024	1.012	1.013
	1.027	1.037	1.033	1.015	1.016
	1.023	1.034	1.034	1.019	1.030
	1.033	1.030	1.030	1.018	1.021
	1.016	1.033	1.035	1.028	1.034
	0.992	1.025	1.016	1.002	0.996
Mean	1.018	1.018	1.027	1.017	1.025
STD	0.0137	0.0126	0.0114	0.0065	0.0100

Table 3.9 Analysis of 1 ppm NH_4-N in water prepared in 20 different flasks following different cleaning methods.

STD = Standard deviation

Flask Number	H_2O	KCl
	------- mg N/litre ------	
1	1.030	1.005
2	1.033	1.009
3	1.021	1.002
4	1.034	1.003
5	1.011	1.006
6	1.019	1.009
7	1.028	1.007
8	1.032	1.005
9	1.025	1.007
10	1.034	1.003
11	1.032	1.002
12	1.021	1.008
13	1.033	1.008
14	1.030	1.004
15	1.013	1.003
16	1.016	1.003
17	1.030	1.001
18	1.021	1.005
19	1.034	1.006
20	0.996	1.003
Mean	1.025	1.005
Standard deviation	0.0100	0.0025

Table 3.10 Analysis of 1 ppm NH_4-N standard solutions in water and KCl prepared in 20 different flasks.

Flask Number	H_2O	KCl
	------- mg N/litre ------	
1	0.992	1.004
2	1.001	1.003
3	1.002	1.007
4	1.002	1.013
5	1.006	1.011
6	1.006	1.021
7	1.004	1.008
8	0.997	1.014
9	1.001	1.002
10	1.004	1.010
11	1.007	1.009
12	1.002	1.013
13	1.011	1.013
14	1.010	1.017
15	1.006	1.007
16	1.001	1.002
17	1.010	1.007
18	1.007	1.005
19	1.009	1.008
20	1.018	1.005
Mean	1.005	1.009
Standard deviation	0.0056	0.0051

Table 3.11 Analysis of 1 ppm NO_3-N standard in water and KCl prepared in 20 different flasks.

CHAPTER FOUR

EFFECT OF THREE PESTICIDES ON SOIL MICROBIAL PROCESSES

4.1 INTRODUCTION

To study the effects of pesticides on soil microbial activities, three commonly used pesticides were chosen. These were captafol, a nonsystemic fungicide, carbaryl, a contact and stomach poison insecticide with slight systemic properties and malathion which is a nonsystemic organophosphorus insecticide and acaricide of low mammalian toxicity.

To evaluate the effects of these three pesticides on soil microbial activities, three biochemical processes, the dehydrogenase and phosphatase activities in soil and nitrification of added ammonium in soils were chosen.

The enzyme dehydrogenase is involved in biological oxidation of soil organic matter. Since the oxidation of organic substrates is coupled with the reduction of molecular oxygen, it is possible to measure the microbial activity by measuring dehydrogenase activity. The phosphatase enzymes are involved in mineralization of organic phosphorus in soil. Measurement of this enzyme activity in soil allows a rapid, effective and cheap means of assessing whether soils have been affected by chemical treatments. Nitrification is a specific process of the oxidation of ammonium to nitrite and nitrite to

nitrate by specific microorganisms. Any effect on these organisms would be reflected in change of nitrification rate. Therefore this process is an indicator of microbial activity in soil.

In order to test the effects of pesticides on soil microbial processes, soil samples can be collected either directly from the field, from plots treated with the desired pesticide or untreated soil can be treated with a known concentration of the pesticide.

Expression of rates of pesticide application to soils has been standardized as kilograms (kg) or litre per hectare (ha). If the specific depth of incorporation of the pesticide is known it is possible to convert application rates as kg or litre/ha to concentrations in the soil as mg/kg.

In the case of foliar application, it is questionable how much pesticide hits the leaf, how much is absorbed by the leaf and how much is washed off. Finally how much pesticide reaches the soil and to what depth the pesticide penetrates the soil is not clear. Even in the case of direct application to the soil pesticides may not be evenly distributed or incorporated the expected depth. Sometimes pesticides applied as dusts may be blown to neighbouring crops and deposited dusts are often vulnerable to wind and rain occurring after application. So it is difficult to compare the effect of pesticides applied under field conditions with pesticide applied at

known rates in laboratory conditions.

When calculating the level of the pesticide in the soil from the application rate it is usually assumed that during application 100% of the product reaches the soil surface. Different authors have assumed different depth of the penetration of a pesticide into the soil, commonly 5 cm depth (Anderson et al., 1987) or 1 cm (Anderson, 1978; Turner, 1979).

In the present study to investigate the effects of pesticides on soil microbial processes, direct application of the pesticide to the soil collected from untreated field sites was chosen. The three pesticides chosen have the recommended doses of 1.7 kg a.i./ha, 0.25 - 2.0 kg a.i./ha and 1.3 - 2.5 kg a.i./ha for captafol, carbaryl and malathion respectively. Assuming the depth of mixing of 1 cm these recommended doses correspond to 17 mg/kg for captafol, 2.5 - 20 mg/kg for carbaryl and 13 - 25 mg/kg for malathion. [1 cm depth of soil is equivalent to 10^8 grams of soil/ha assuming an average bulk density for soil of 1.0].

As the effects of pesticides differ depending on application rate, two levels of application were chosen. The same rates were chosen for all three pesticides, a low rate 10 mg/kg which is within the range of recommended doses and a high rate 100 mg/kg which is 10 times that low rate.

4.2 METHODS

4.2.1 EXPERIMENTAL DESIGN

Each experiment consisted of two soils Darvel and Dreghorn Series treated with 3 pesticides (captafol, carbaryl and malathion) at two levels (10 mg/kg soil and 100 mg/kg soil) along with an untreated control for each soil making a total of 14 treatments.

4.2.2 TREATMENT OF SOIL WITH PESTICIDES

Pesticides were applied to the soils as described in the Section 2.7. Fresh soil samples treated with pesticides were prepared separately for each experiment and then stored at 2°C if not used at once.

4.2.3 PHOSPHATASE ACTIVITY

Each of the 14 treatments was replicated 5 times making a total of 70 determinations. In order to cope with the large number of determinations the experiment was split into two batches for each soil. The two batches of the experiment were as follows.

1st batch ——— Untreated soil (control)
Captafol low level
Captafol high level

2nd batch ——— Untreated soil (control)
Carbaryl low level
Carbaryl high level
Malathion low level
Malathion high level

There was a control treatment in both batches.

In the case of the phosphatase activity experiment, the first batch of the experiment was analysed in the morning and the other batch in the afternoon of the same day.

4.2.4 DEHYDROGENASE ACTIVITY

The experiment was carried out in two batches as described for the phosphatase activity but the first batch of the samples were analysed one day and the other batch on the following day for each soil. The dehydrogenase activity was measured within the week after measuring phosphatase activity of the pesticide treated soils which were stored in cold room at 2°C.

4.2.5 NITRIFICATION

A randomized block design was used in this experiment and was replicated 3 times with the 14 treatments making a total of 42 incubations. Both soils were incubated in 3 chambers as described in the Section 2.10. After taking a sample for 0 days incubation, samples were taken

after the following periods of incubation 2, 5, 7, 9 and 11 days.

Samples were extracted with 0.5M K_2SO_4 as described in the Section 2.11.4. The filtrates obtained were analysed for ammonium, nitrate and nitrite nitrogen by the Technicon AutoAnlyzer II using methods as described in Sections 2.12.1 and 2.12.2.

4.3 RESULTS AND DISCUSSION

4.3.1 PHOSPHATASE AND DEHYDROGENASE ACTIVITIES

For the assay of each enzyme activity it was necessary to split the experiment into two parts for each soil because of the large numbers of determinations. There were significant differences between the phosphatase activities of the control treatments run with each part of the experiment for both Darvel and Dreghorn soils (Tables 4.1 and 4.2). The differences were significant at the 0.1% level for Darvel soil and at the 1% level for Dreghorn soil. Due to this variation among the controls of the different batches of the experiment, it was not possible to analyse the results for the whole experiment by using analysis of variance. Therefore, a pooled t test was used to compare each treatment with the corresponding untreated control to determine if the pesticide treatment used affected the enzyme activity.

This variability between the controls was unexpected. For the determination of phosphatase activity, the same batches of reagents, same incubator and same spectrophotometer were used for both parts of the experiment for each soil. The length of time to set up each part of the experiment and the addition of NaOH to stop the reaction may have been slightly different in each batch because the second batch contained more samples. This variation in time to set up the experiment

could make small differences to the measured enzyme activity. The experiment was set up at room temperature approximately 20°C which is lower than the temperature of incubation at 37°C. The rate of enzyme activity should be much lower at 20°C than at 37°C. Therefore, small differences in time taken to set up and stop the assay compared with the 1 hour of incubation at 37°C could not account for the differences in phosphatase activity observed between the two sets of controls.

The reason for the variability between the controls of the different batches cannot be explained. However, within each batch all the samples were treated identically and replication among controls was good (Tables 4.1 and 4.2). The standard deviations presented in Tables 4.3 and 4.4 also show good replication within treatments. Therefore, differences between treatments run together within a batch are valid and can be tested using a pooled t test.

	Phosphatase activity µ moles/g/h	
	Batch 1	Batch 2
	1.50	1.65
	1.50	1.65
	1.53	1.67
	1.53	1.65
	1.59	1.62
Mean	1.53	1.65 ***

Table 4.1 Phosphatase activity of untreated (control) Darvel soil measured on two occasions.

*** Significantly different at 0.1% level using a pooled t test.

	Phosphatase activity μ moles/g/h	
	Batch 1	Batch 2
	1.64	2.18
	1.70	2.18
	1.76	1.99
	1.64	1.80
	1.64	1.80
Mean	1.68	1.99 **

Table 4.2 Phosphatase activity of untreated (control) Dreghorn soil measured on two occasions.

** Significantly different at 1% level using a pooled t test.

Treatment	Phosphatase activity (μ moles/g/h) mean	SD[b]	
Control	1.53	0.037	
Captafol 10 mg/kg	1.48	0.100	NS
Captafol 100 mg/kg	1.42	0.034	**
------------------	----	-----	---
Control	1.65	0.018	
Carbaryl 10 mg/kg	1.47	0.078	**
Carbaryl 100 mg/kg	1.44	0.068	***
Malathion 10 mg/kg	1.53	0.055	**
Malathion 100 mg/kg	1.47	0.113	**

Table 4.3 Effect of pesticides on phosphatase activity of Darvel soil.

b Standard deviation.

* Significantly different from the control at 5% level using a pooled t test.

** Significant at 1% level.

*** Significant at 0.1% level.

ns not significant.

Treatment		Phosphatase activity (μ moles/g/h) mean	SD[b]	
Control		1.68	0.053	
Captafol	10 mg/kg	1.51	0.034	***
Captafol	100 mg/kg	1.50	0.059	**
Control		1.99	0.190	
Carbaryl	10 mg/kg	1.96	0.199	ns
Carbaryl	100 mg/kg	1.81	0.050	ns
Malathion	10 mg/kg	1.78	0.050	*
Malathion	100 mg/kg	1.74	0.046	*

Table 4.4 Effect of pesticides on phosphatase activity of Dreghorn soil.

b Standard deviation.

* Significantly different from control at 5% level using a pooled t test.

** Significant at 1% level.

*** Significant at 0.1% level.

ns not significant.

Significant differences were also found between the dehydrogenase activities of the control treatments run with each part of the experiment for Dreghorn soil only (Table 4.6). The differences were significant at the 0.1% level. The other soil, Darvel, did not give significant differences between the dehydrogenase activities of the control treatments (Table 4.5). As with phosphatase for the determination of dehydrogenase activity, the same batch of reagents, same incubator and same spectrophotometer were used in both batches of determinations. So there should not be such variation between the two sets of controls for the two batches. Long exposure to visible light causes a colour change of triphenyl formazan from red to yellow but the reaction is reversed in the dark. Short exposure to visible light during filtration may have caused some variability as there is a possibility of differences in light intensity on different days. Replication within the control treatments and treated soils was good as shown in Tables 4.5, 4.6, 4.7 and 4.8. So a pooled t test comparing the treated soils with the corresponding untreated control run in the same batch, is a valid way to interpret the results.

Dehydrogenase activity ($\mu g/g/h$)		
	Batch 1	Batch 2
	6.31	6.79
	6.31	6.63
	6.47	6.79
	6.79	6.79
	6.63	6.63
Mean	6.50	6.73 ns

Table 4.5 Dehydrogenase activity of untreated (control) Darvel soil measured on two occasions.

ns not significantly different.

	Dehydrogenase activity ($\mu g/g/h$)	
	Batch 1	Batch 2
	3.99	3.65
	3.82	3.65
	3.99	3.65
	4.08	3.65
	4.17	3.65
Mean	4.01	3.65 ***

Table 4.6 Dehydrogenase activity of untreated (control) Dreghorn soil measured on two occasions.

*** Significantly different at 0.1% level using a pooled t test.

Treatment		Dehydrogenase activity (μg/g/h) mean	SD[b]	
Control		6.50	0.209	
Captafol	10 mg/kg	5.69	0.283	***
Captafol	100 mg/kg	3.25	0.126	***
-------------------		----	-----	---
Control		6.73	0.088	
Carbaryl	10 mg/kg	6.16	0.110	***
Carbaryl	100 mg/kg	6.06	0.212	***
Malathion	10 mg/kg	6.31	0.110	***
Malathion	100 mg/kg	5.90	0.088	***

Table 4.7 Effect of pesticides on dehydrogenase activity of Darvel soil.

b Standard deviation.

*** Significantly different from the control at 0.1% level using a pooled t test.

Treatment		Dehydrogenase activity (μg/g/h) mean	SD[b]	
Control		4.01	0.130	
Captafol	10 mg/kg	3.78	0.116	*
Captafol	100 mg/kg	2.29	0.076	***
----------	----------	----	-----	---
Control		3.65	0.000	
Carbaryl	10 mg/kg	3.63	0.040	ns
Carbaryl	100 mg/kg	3.39	0.060	***
Malathion	10 mg/kg	3.40	0.156	**
Malathion	100 mg/kg	3.37	0.093	***

Table 4.8 Effect of pesticides on dehydrogenase activity of Dreghorn soil.

b Standard deviation.

* Significantly different from the control at 5% level using a pooled t test.

** Significant at 1% level.

*** Significant at 0.1% level.

ns not significant.

From the Table 4.3 it can be seen that all three pesticides used significantly reduced the phosphatase activity of Darvel soil (7 to 13%) at both low level application and 10 times that level, except for the low level of captafol. Captafol at the low level did not give any significant effect on Darvel soil.

It can be seen from the Table 4.4 that captafol and malathion significantly reduced the phosphatase activity of Dreghorn soil (10 - 13%) at both levels of application. Carbaryl did not give any significant effect on Dreghorn soil at either level of application. There was no clear effect of the application rate of any of the three pesticides used in case of phosphatase activity.

Table 4.7 shows that all three pesticides, captafol, carbaryl and malathion at both levels of application significantly reduced the dehydrogenase activity of Darvel soil by 6 to 50%.

For Dreghorn soil, of the three low level pesticide applications, only carbaryl did not result in a significant difference from the control (Table 4.8). But all other low and high levels of application of the three pesticides used significantly reduced the dehydrogenase activity of Dreghorn soil by 6 to 43%. The level of application of the pesticides had a clear effect on the dehydrogenase activity of both soils especially in the case of captafol. Thus captafol at the low level

inhibited the dehydrogenase activity by 6 and 13% and at high level 43 and 50% respectively for Dreghorn and Darvel soils.

Results from the literature show considerable diversity in the effect of pesticides on phosphatase and dehydrogenase activity of soil.

Simazine at the rate of 200 kg ha^{-1} applied to a chernozem soil inhibited the phosphatase activity and the rate of 4 kg ha^{-1} in the same soil increased the activity (Yurkevich and Tolkachev, 1972). The same product applied at the rate of 2 kg ha^{-1} to a gray forest silty medium loam soil (Boiko et al., 1969) and at 6 to 12 kg ha^{-1} in leached loam chernozem soil increased the phosphatase activity (Arshidinov et al., 1974).

Atrazine at the rate of 4 and 10 kg ha^{-1} applied to a heavy loam chernozem soil did not affect the phosphatase activity (Pilipets and Litvinov, 1972) whereas the same product at the rate of 4 kg ha^{-1} in a loamy sand soil inhibited the enzyme activity (Voets et al., 1974).

2,4-D at the rate of 0.4 kg ha^{-1} applied to a pale sandy loam chernozem soil did not affect the phosphatase activity (Chulakov and Zharasov, 1973) but this compound at the rate of 2 and 100 kg ha^{-1} applied to a chernozem soil decreased the enzyme activity (Yurkevich and Tolkachev, 1972).

Lenacil at the rate of 1.2 to 1.7 kg ha^{-1} had no effect on phosphatase activity when applied to dark

chestnut heavy loam and pale chestnut light loam soil (Chulakov and Zharasov, 1973).

Other herbicides which have been reported to decrease the phosphatase activity include pyrazon (Voets and Verstraete, 1973) and lenacil at 1 kg ha^{-1} in combination with dichloral urea (DCU) at 5 kg ha^{-1} (Barona, 1974).

The herbicide diuron at the rate of 6 kg ha^{-1} applied to a leached loam chernozem soil increased the phosphatase activity (Arshidinov et al., 1974).

Other pesticides including a fungicide 2-(thiocyanomethylthio)benzothiazole (TCMTB) at the rate of 5 to 30 kg ha^{-1} (Voets and Vandamme, 1970) and insecticides such as malathion, chlordane, aldrine and lindane at concentration of 0.02 mol have been reported to inhibit phosphatase activity of soil (Komeil, 1986). But chlordane at the rate of 5 $\mu g\ g^{-1}$ applied to an organic soil increased enzyme activity (Tu, 1981).

The work reported here was to determine the effect of three commonly used pesticides on soil enzyme activity. The insecticide malathion at both levels of application significantly inhibited the phosphatase activity of both Darvel and Dreghorn soils (Tables 4.3 and 4.4). The concentration-dependent effect of this insecticide was studied under laboratory conditions by Komeil (1986). He found that at 0.02 mol the percentage of inhibition was 96% by this insecticide.

The other insecticide carbaryl at both levels of

application also significantly reduced the phosphatase activity of Darvel soil but did not affect this enzyme activity significantly in Dreghorn soil (Tables 4.3 and 4.4) at either level of application. In a study of the effects of organophosphorus and carbamate insecticides on enzymic activities in the soil environment Hong and Kim (1986) found that the inhibitory effects of insecticides in soil decreased in the following order: dithiophosphoric acid > thiophosphoric acid > carbamate insecticides for phosphatase enzyme when the soil treated with urea was incubated at 28°C for 56 days.

The fungicide captafol used in this experiment at both levels of application significantly reduced the phosphatase activity of Dreghorn soil (Table 4.4) and at high level only this compound also inhibited the phosphatase activity of Darvel soil (Table 4.3). In previous work Voets and Vandamme (1970) using another fungicide TCMTB at the rate of 5 to 30 kg ha^{-1} found that this compound inhibited the phosphatase activity in proportion to the concentration of the compound applied to an arable soil.

Most workers consider dehydrogenase activity to be a measure of total respiratory activity. One application of atrazine to a cultivated field at the normal field rate and two applications to a bare soil, were enough to increase the dehydrogenase activity. The use of the same product for fifteen years, in apple orchards decreased

all the enzymatic activity (Voets et al., 1974). On the other hand, benomyl remained without effect on the activity of dehydrogenases (van Fassen, 1974).

In regard to dehydrogenase activity in general, a range of results is possible, from no effect with simazine at normal rate (Kulinska, 1967) and chloroxuron at the rate of 1 - 10 kg ha^{-1} (Odu and Horsfall, 1971), to inhibition with rates of tribunil 3 kg ha^{-1}, trichloroacetal dehydrate 20 to 50 litre ha^{-1} and wolfen-thiuram 2 kg ha^{-1} (Hickisch et al., 1984), roundup at recommended and excessive rates (Schuster et al., 1989), sodium chlorate at 150 kg ha^{-1} (Karki et al., 1973) and atrazine at the rate of 0.5 and 5 g/100 g of soil (El-Din et al., 1977) and to stimulation with parathion methyl at rate equivalents of 15 kg a.i. ha^{-1} or lower (Naumann, 1970a).

Malathion at both levels of application was found to inhibit the dehydrogenase activity of both Darvel and Dreghorn soils used in this experiment (Table 4.7 and 4.8). Carbaryl at both levels of application also significantly reduced the dehydrogenase activity of Darvel soil (Table 4.7) but only the high rate reduced the enzyme activity significantly in case of Dreghorn soil (Table 4.8). Carbaryl has been reported to prevent the dehydrogenase accumulation in a flooded soil at the rate of 1 - 100 $\mu g/g$ (Chendrayen and Sethunathan, 1980).

The fungicide captafol at both levels of application significantly inhibited the dehydrogenase activity of both Darvel and Dreghorn soils used in this experiment (Tables 4.7 and 4.8). This finding is contrary to findings in previous work by Naumann (1970b) with other fungicide captan, which in concentrations of 250 ppm first stimulated dehydrogenase activity but after 16 weeks the activity was identical with that in untreated soil.

On the other hand, addition of benomyl at 1 to 100 μg/g in flooded soil prevented the dehydrogenase accumulation (Chendrayan and Sethunathan, 1980). The effect of the fungicide Dexon on dehydrogenase activity was also found to be inhibitory and the inhibition was related to the fungicide concentration and varied with incubation time (Karanth et al., 1975).

Thus the range of pesticide action on soil enzymes is clearly indicated that the effect of the pesticide depends on its concentration, type of soil and the time of incubation.

4.3.2 NITRIFICATION

The results of the nitrification experiment are presented in Figures 4.1 - 4.14. The analysis of variance showed NO_2-N levels were not significantly affected by the pesticide treatment. Accumulation of NO_2-N was smaller then 2 μg/g in the case of Darvel soil and it

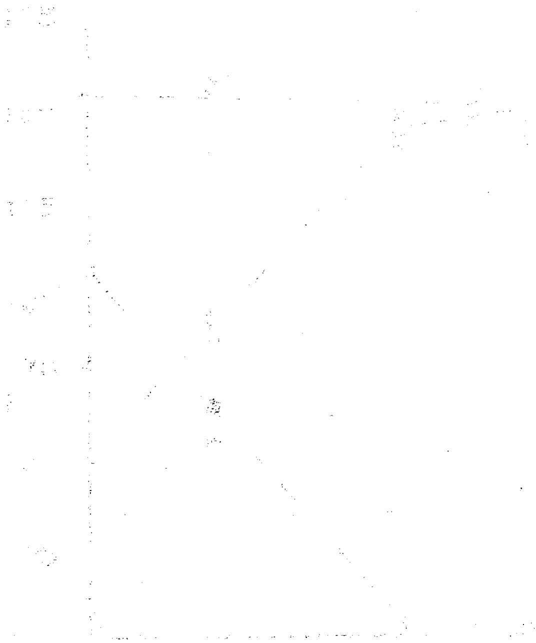
was smaller than 1 μg/g in the case of Dreghorn soil. So NO_2-N is not plotted in the graphs.

There was some variability in total inorganic nitrogen but there was no trend of decrease or increase in total inorganic nitrogen. Therefore the added NH_4-N was not immobilized nor NH_4-N was produced by mineralization of soil organic matter.

The disappearance of NH_4-N and the accumulation of NO_3-N were linear except in the case on malathion high level in Darvel soil. No lag period was observed in any case. Darvel soil nitrified faster than expected. Only 3 points were found on the line. Some curvature, particularly in the control suggests that the third point is not on the line because nitrification was completed before the sampling on day 5.

The rate of nitrification was the preferred measure of effects of pesticides on soil nitrification. The slope and accumulation of NO_3-N were linear in the case of Dreghorn soil, but this is questionable in the case of Darvel soil due to the lack of points. For this reason the results of NO_3-N levels of day 2 for Darvel soil are presented (Table 4.9). In the case of Dreghorn soil there was no such problem of lacking points but the results of NO_3-N levels of day 7 for Dreghorn soil are presented (Table 4.10). Differences between treatment means and controls were tested by a multiple comparison procedure (Fisher, LSD).

Nitrification rates were calculated by using linear regression to attain the best fit straight line to the linear part of the nitrate accumulation graphs. These nitrification rates are presented in Tables 4.11 and 4.12.



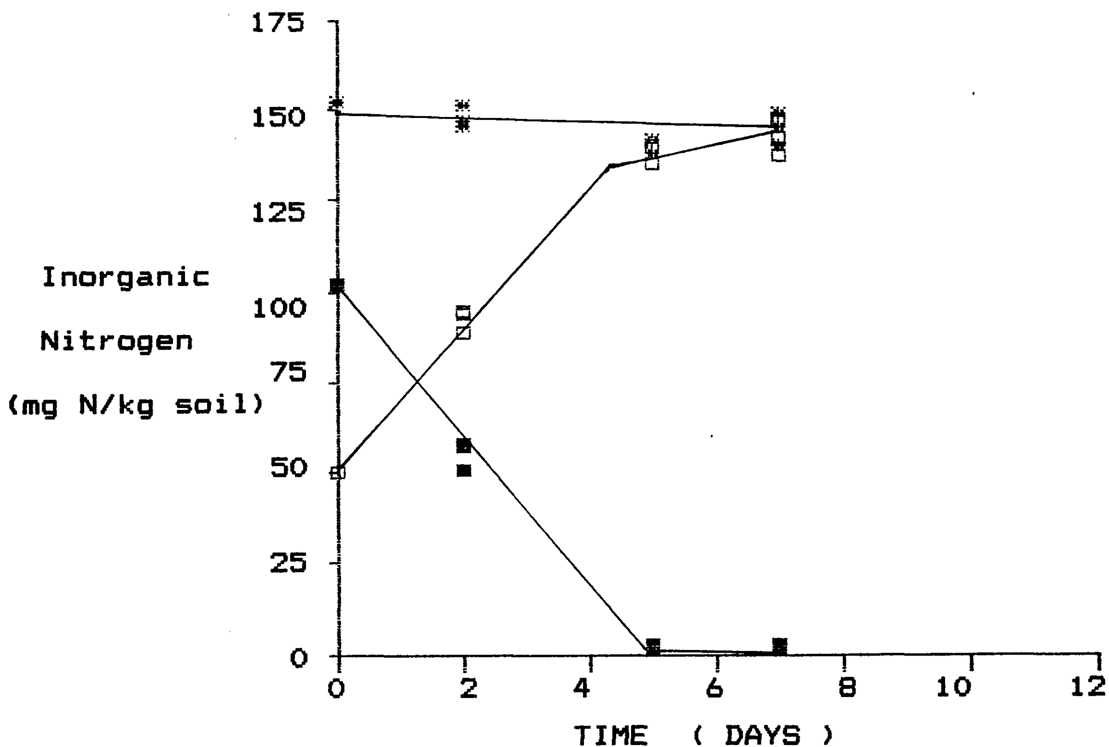


FIG 4.1 Nitrification in untreated Darvel soil.

(*) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

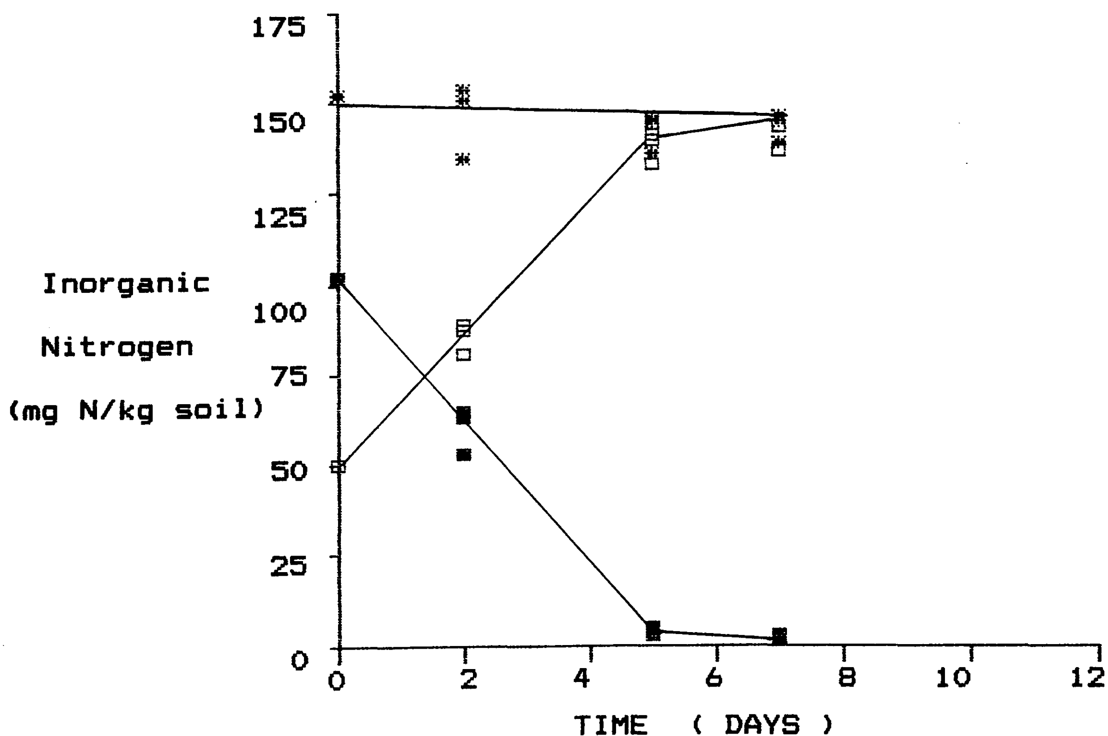


FIG 4.2 Nitrification in Darvel soil treated with captafol at 10 mg/kg soil.

(*) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

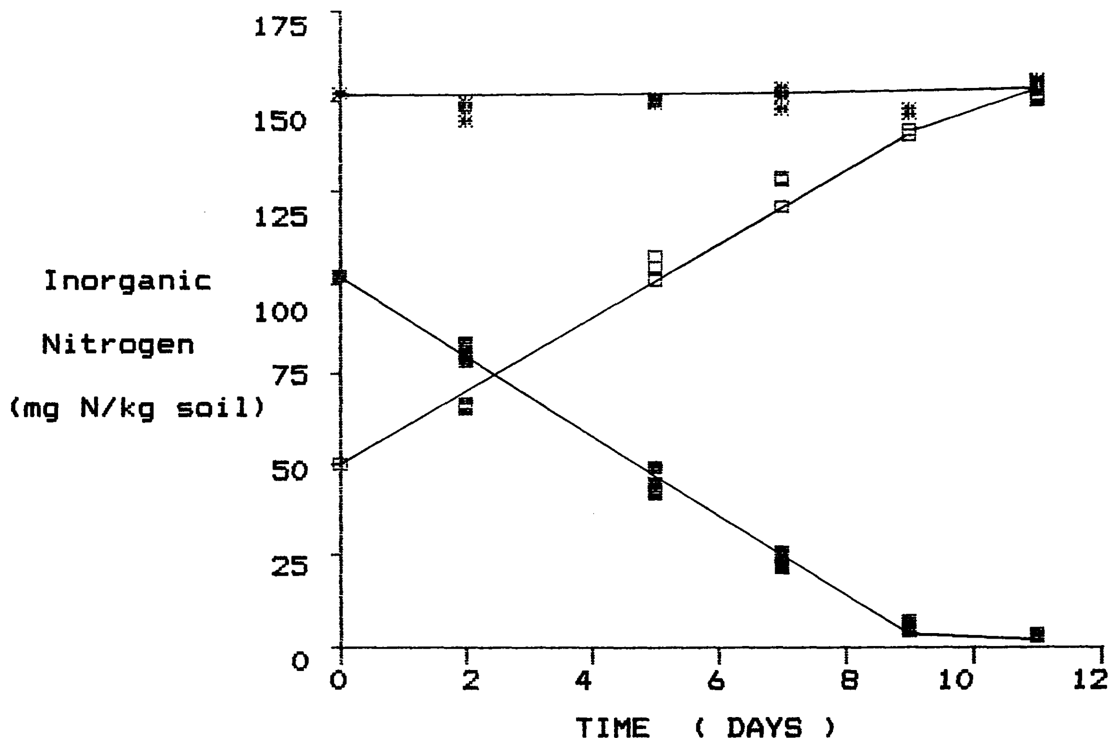


FIG 4.3 Nitrification in Darvel soil treated with captafol at 100 mg/kg soil.

(*) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

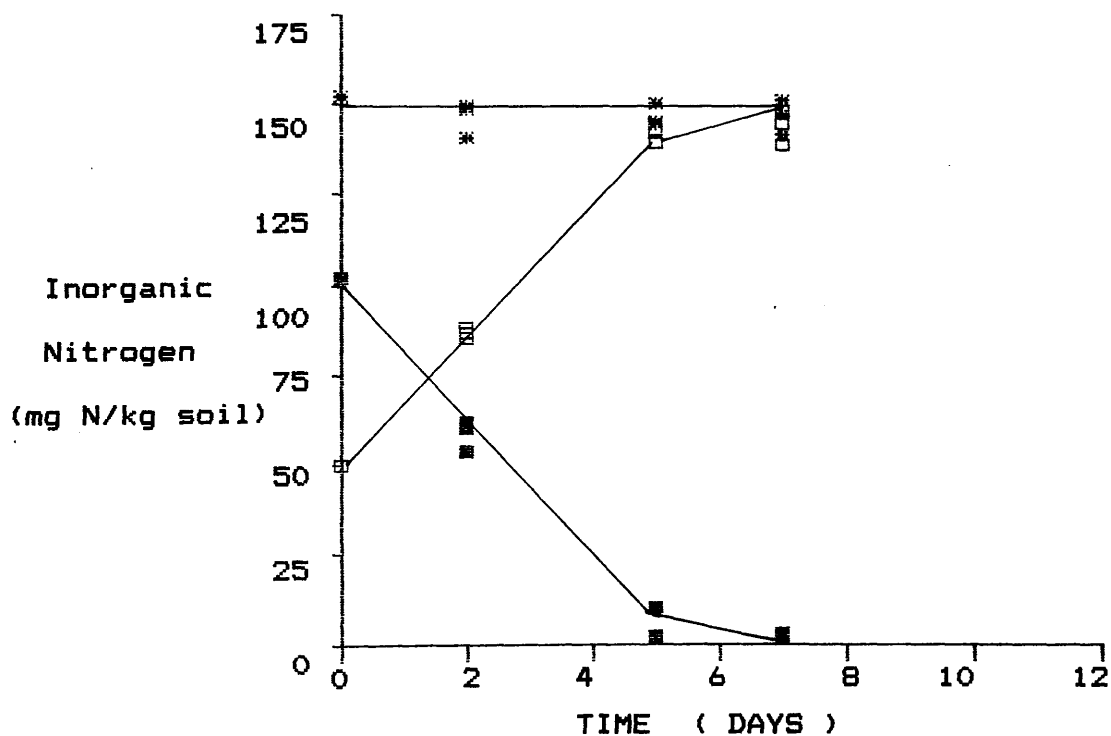


FIG 4.4 Nitrification in Darvel soil treated with carbaryl at 10 mg/kg soil.

(*) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

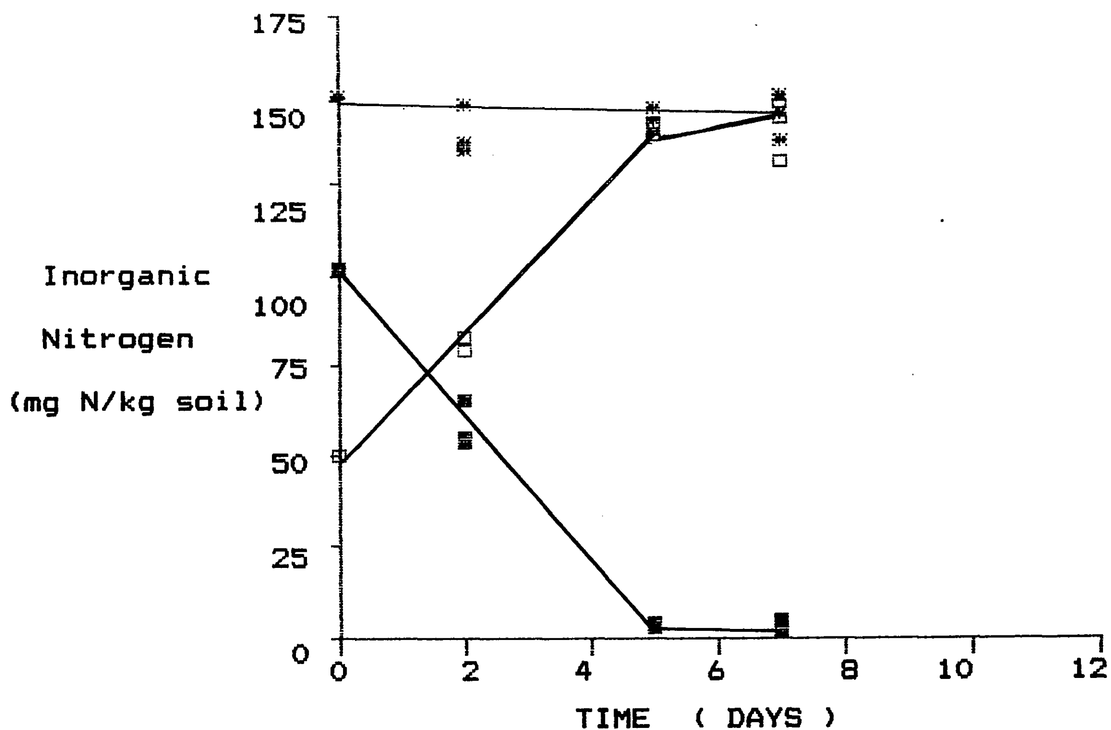


FIG 4.5 Nitrification in Darvel soil treated with carbaryl at 100 mg/kg soil.

(*) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

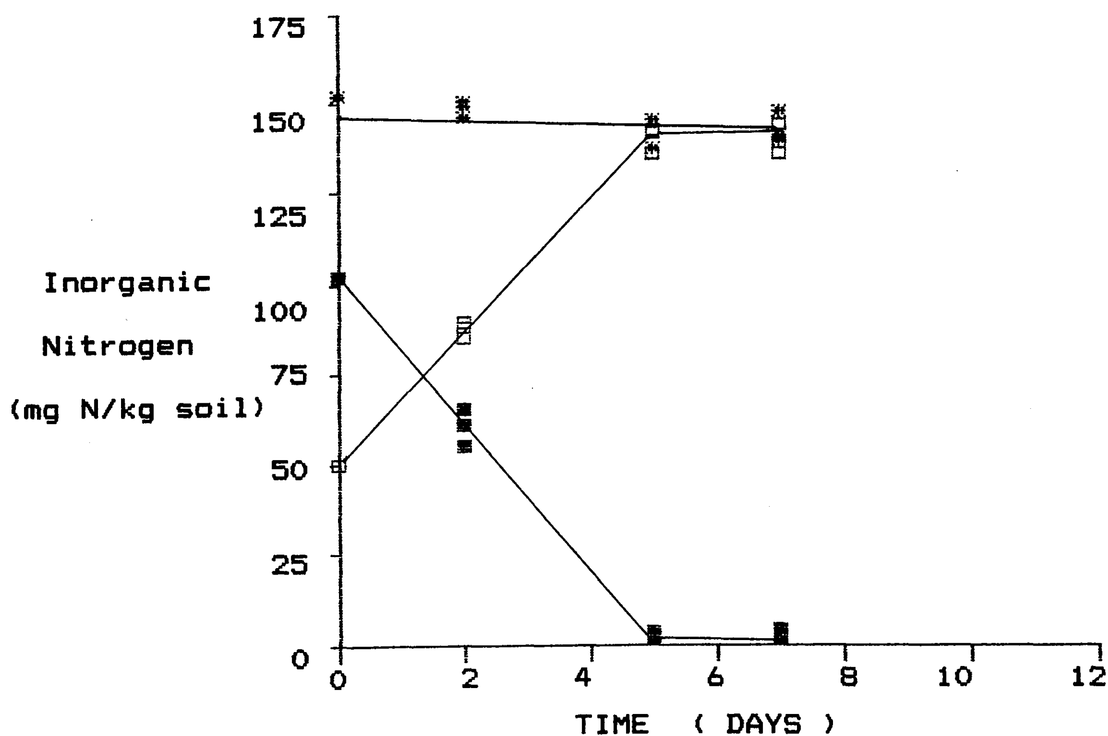


FIG 4.6 Nitrification in Darvel soil treated with malathion at 10 mg/kg soil.

(*) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

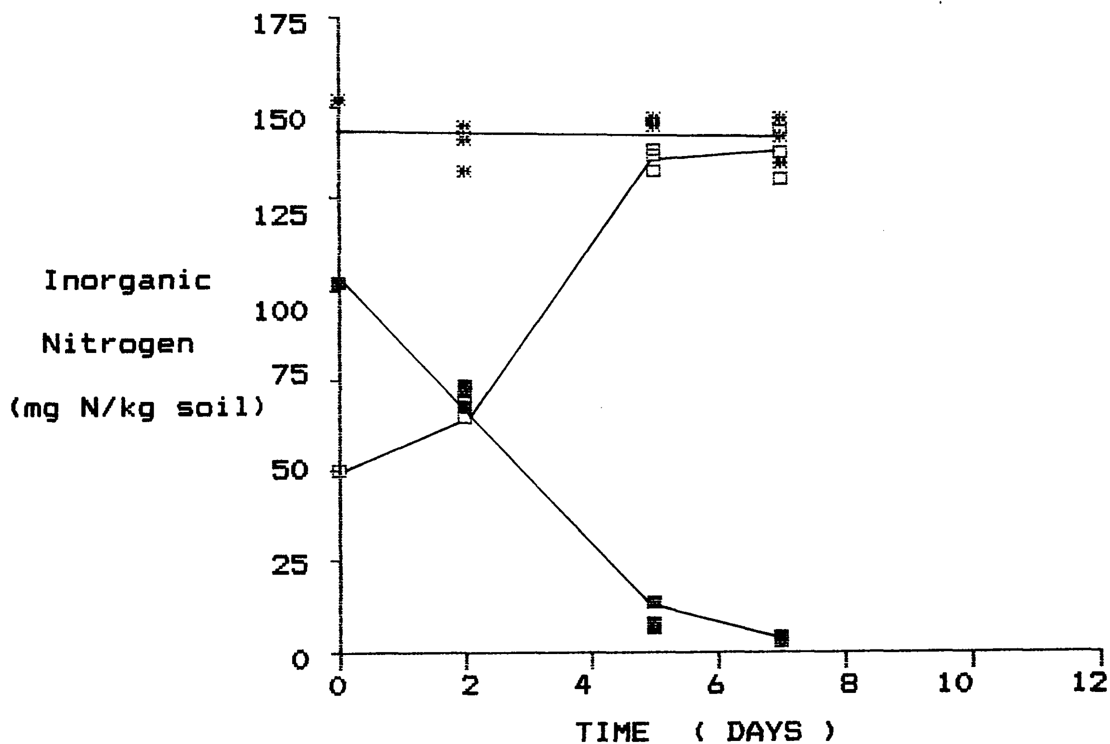


FIG 4.7 Nitrification in Darvel soil treated with malathion at 100 mg/kg soil.

(*) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

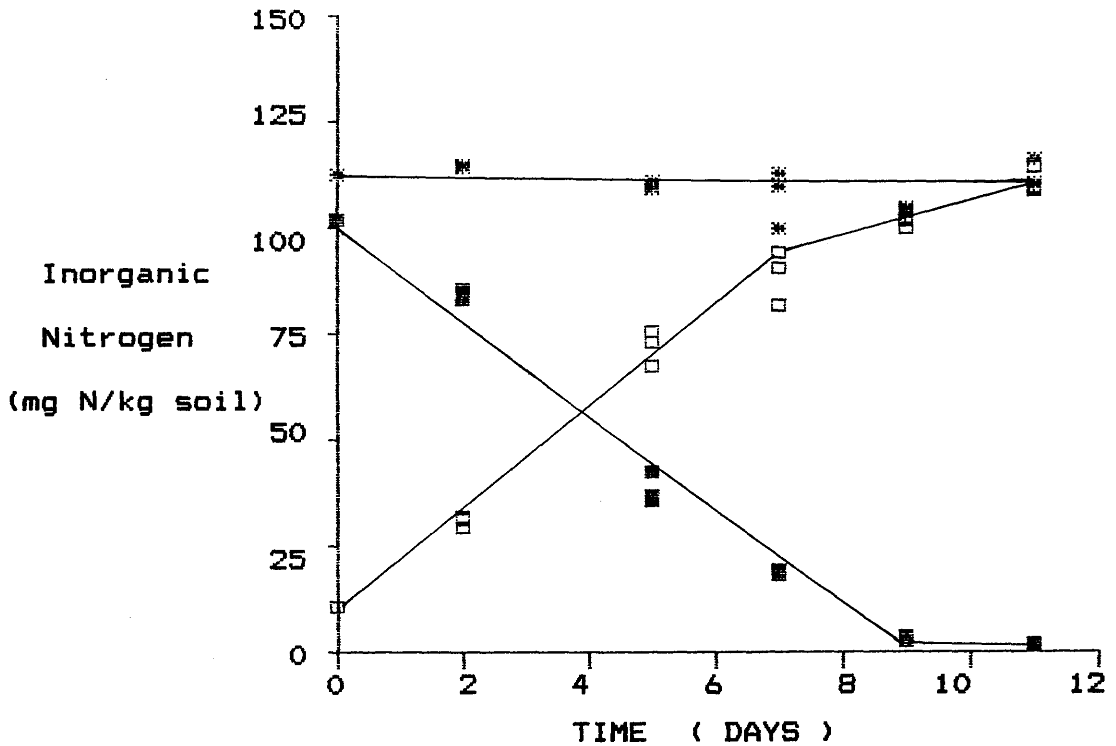


FIG 4.8 Nitrification in untreated Dreghorn soil.

(✱) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

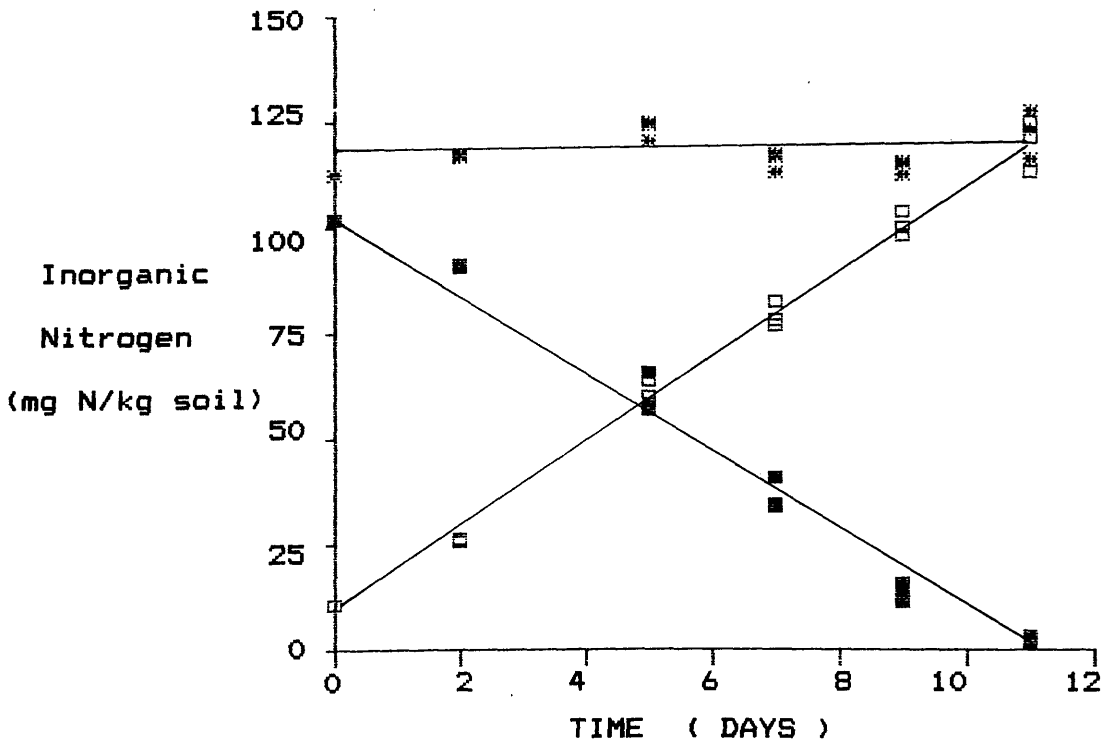


FIG 4.9 Nitrification in Dreghorn soil treated with captafol at 10 mg/kg soil.

(✱) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

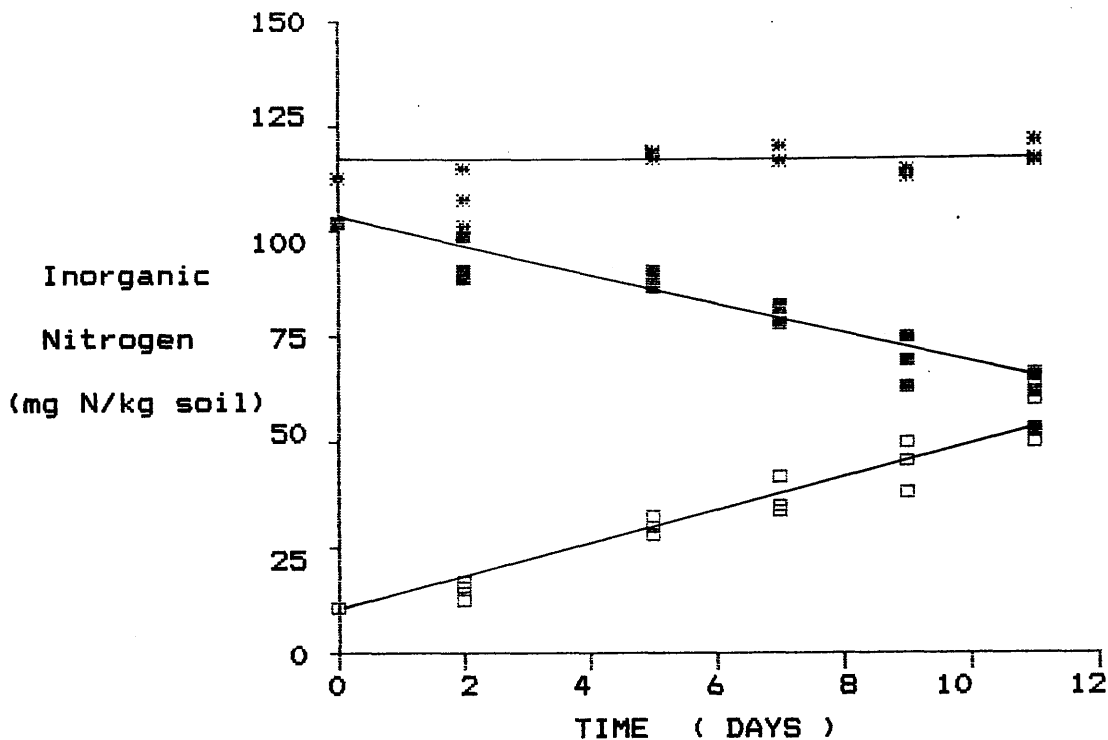


FIG 4.10 Nitrification in Dreghorn soil treated with captafol at 100 mg/kg soil.

(*) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

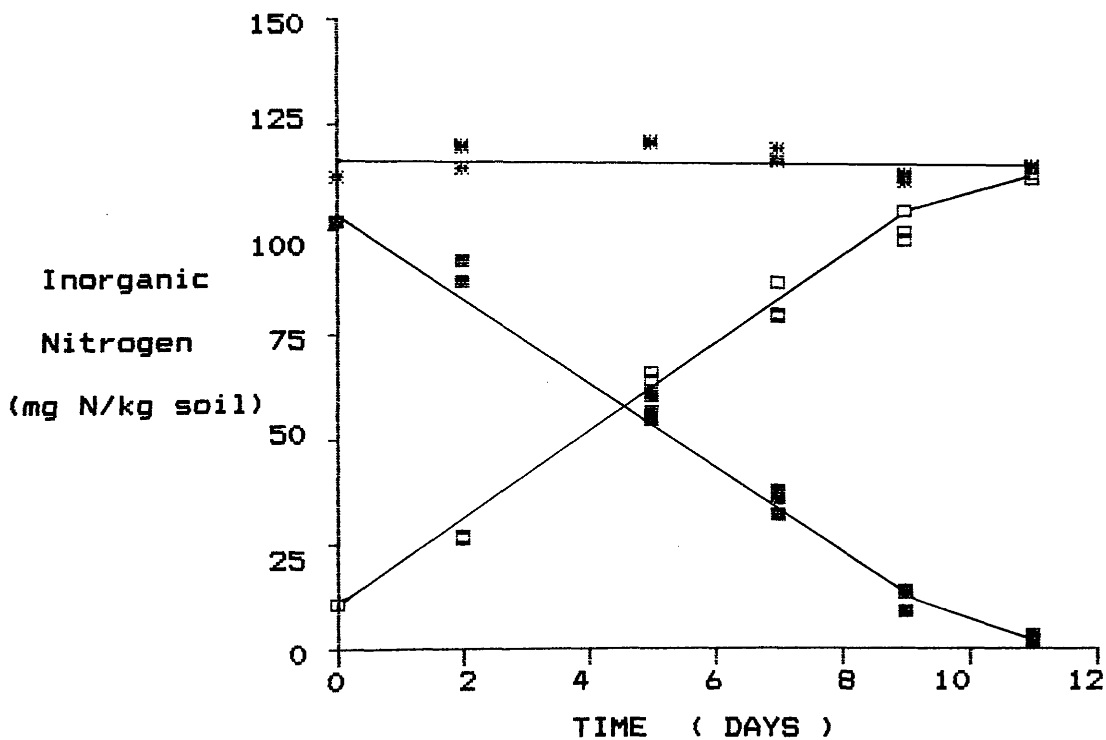


FIG 4.11 Nitrification in Dreghorn soil treated with carbaryl at 10 mg/kg soil.

(*) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

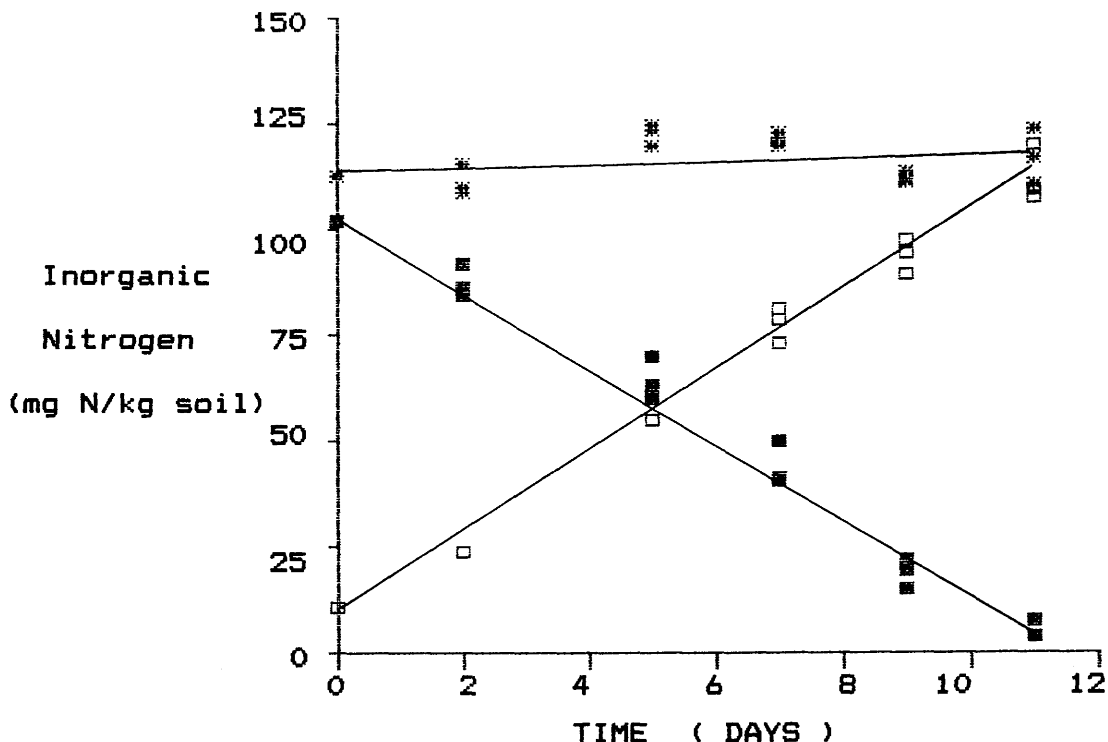


FIG 4.12 Nitrification in Dreghorn soil treated with carbaryl at 100 mg/kg soil.

(✱) Total inorganic – N
(■) Ammonium – N
(□) Nitrate – N

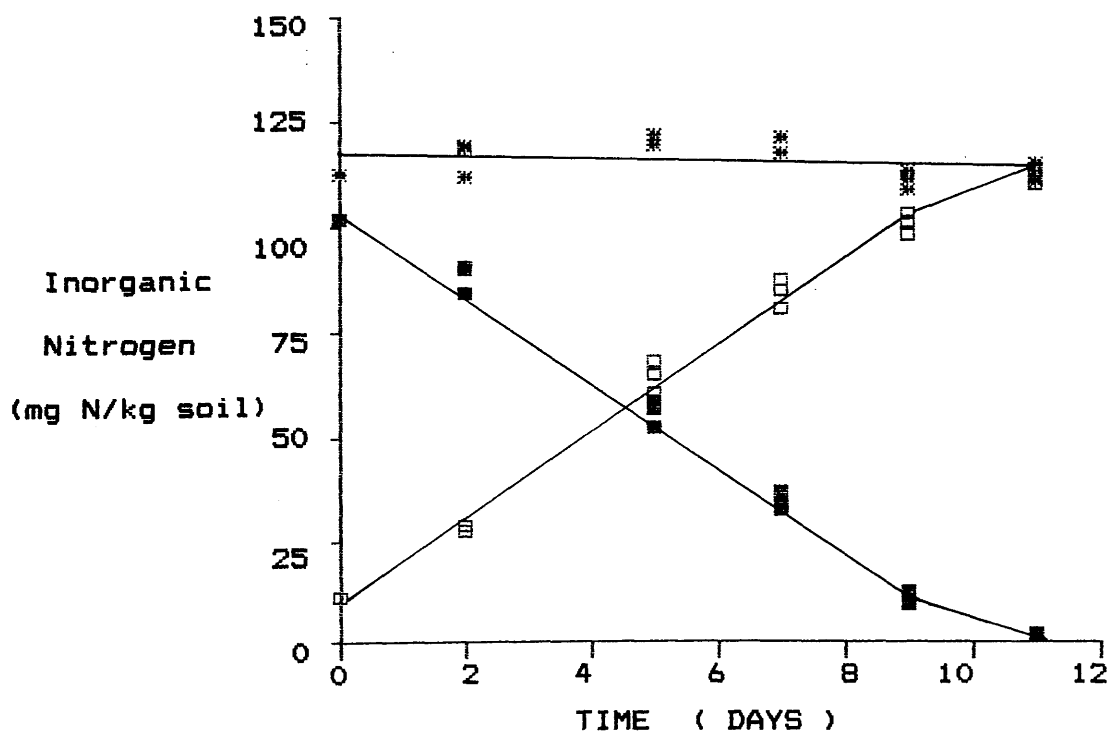


FIG 4.13 Nitrification in Dreghorn soil treated with malathion at 10 mg/kg soil.

(✱) Total inorganic – N
(■) Ammonium – N
(□) Nitrate – N

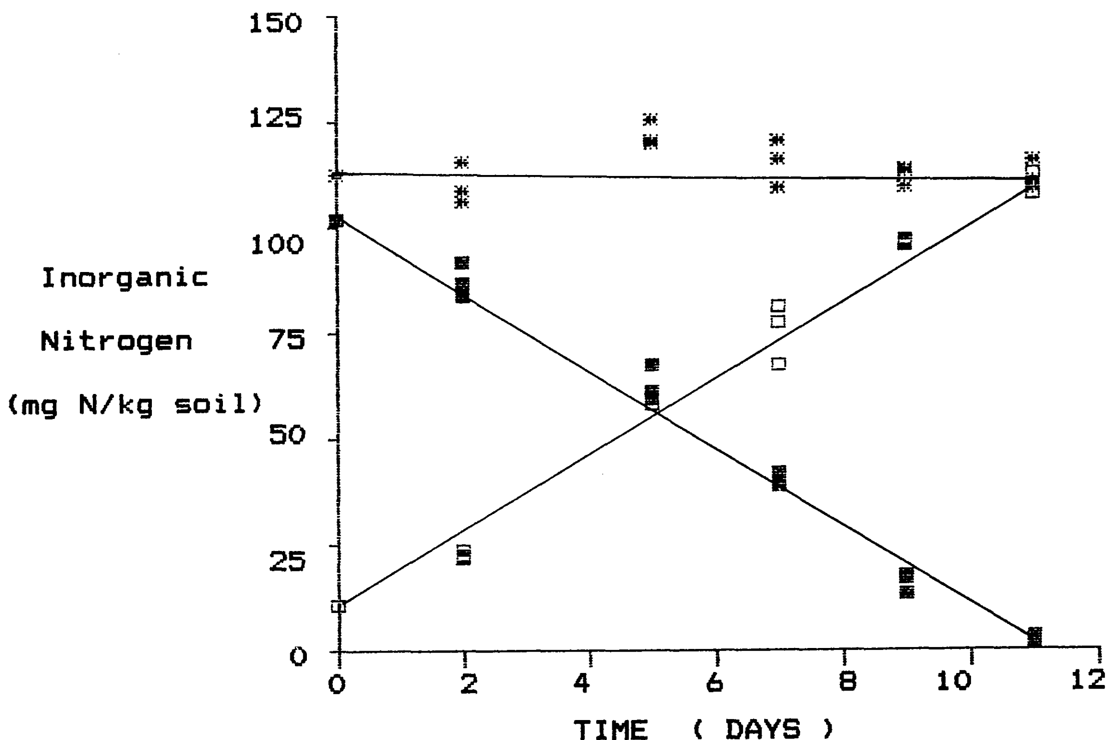


FIG 4.14 Nitrification in Dreghorn soil treated with malathion at 100 mg/kg soil.

(✱) Total inorganic - N
(■) Ammonium - N
(□) Nitrate - N

Treatment		Nitrate-N ($\mu g/g$) mean	Difference from control	
Control		92.65		
Captafol	10 mg/kg	85.97	6.68	**
Captafol	100 mg/kg	66.99	25.66	***
Carbaryl	10 mg/kg	87.41	5.24	*
Carbaryl	100 mg/kg	82.22	10.43	***
Malathion	10 mg/kg	88.35	4.30	ns
Malathion	100 mg/kg	68.85	23.80	***

Table 4.9 Effect of pesticides on NO_3-N production by Darvel soil at day 2 of incubation

ns not significant

* significant at 5% level using Fisher, LSD

** significant at 1% level

*** significant at 0.1% level

Treatment		Nitrate-N ($\mu g/g$) mean	Difference from control	
Control		88.81		
Captafol	10 mg/kg	79.10	9.71	*
Captafol	100 mg/kg	36.83	51.98	***
Carbaryl	10 mg/kg	81.62	7.19	ns
Carbaryl	100 mg/kg	77.46	11.35	*
Malathion	10 mg/kg	84.30	4.51	ns
Malathion	100 mg/kg	75.27	13.54	**

Table 4.10 Effect of pesticides on NO_3-N production by Dreghorn soil at day 7 of incubation

ns not significant

* significant at 5% level using Fisher, LSD

** significant at 1% level

*** significant at 0.1% level

Treatment		Nitrification rate (µg N/g soil/day) mean	Difference from control	
Control		17.48		
Captafol	10 mg/kg	17.64	0.16	ns
Captafol	100 mg/kg	10.70	6.78	***
Carbaryl	10 mg/kg	18.32	0.84	ns
Carbaryl	100 mg/kg	18.34	0.86	ns
Malathion	10 mg/kg	18.03	0.55	ns
Malathion	100 mg/kg	17.67	0.19	ns

Table 4.11 Effect of pesticides on nitrification rate in Darvel soil

ns not significant

*** significant at 0.1% level, using Fisher LSD

Treatment		Nitrification rate (μg N/g soil/day) mean	Difference from control	
Control		10.50		
Captafol	10 mg/kg	10.17	0.33	ns
Captafol	100 mg/kg	4.24	6.26	***
Carbaryl	10 mg/kg	10.21	0.29	ns
Carbaryl	100 mg/kg	9.48	1.02	*
Malathion	10 mg/kg	10.32	0.18	ns
Malathion	100 mg/kg	9.44	1.06	*

Table 4.12 Effect of pesticides on nitrification rate in Dreghorn soil

ns not significant

* significant at 5% level using Fisher, LSD

*** significant at 0.1% level

From the nitrification rate and the levels of NO_3-N produced by day 2 of the incubation of Darvel soil, captafol at 100 mg/kg significantly reduced the conversion of NH_4-N to NO_3-N (Table 4.9). Other levels of all three pesticides except malathion low level also significantly reduced the nitrification rate of Darvel soil but these differences were not observed in the nitrification rates shown in Table 4.11. As there are no lack of points on the line and some curvature in the control the results presented in Table 4.9 are considered more reliable than those presented in Table 4.11.

In the case of Dreghorn soil results of the NO_3-N levels of day 7 presented in Table 4.10 and the nitrification rate presented in Table 4.12 show a good agreement. Both slopes and NO_3-N levels of day 7 agreed a clear effect of captafol high level which significantly reduced the nitrification rate of Dreghorn soil. From the Table 4.12 it can be seen that the high levels of all three pesticides captafol, carbaryl and malathion significantly reduced the nitrification rate. But the low levels of all three pesticides did not affect the nitrification rate significantly. Though high levels of all three pesticides significantly reduced the nitrification process captafol had a very large effect in comparison to other two pesticides.

Various studies have shown a diversity in the effects of pesticides on nitrification in soil. Captan, thirum

and verdasan at concentrations of 0.5 - 10, 0.5 - 5 and 0.1 - 0.5 kg ha^{-1} respectively applied to a grass soil either stimulated or had no effect on nitrification. At higher rates, captan (25 kg ha^{-1}), thiram (10 and 25 kg ha^{-1}) and verdasan at 1 - 5 kg ha^{-1} inhibited the process (Wainwright and Pugh, 1973). Dexon in an unspecified soil inhibited nitrification at 35 kg ha^{-1} but not at lower rates of 1.1 to 1.4 kg ha^{-1} (Agnihotri, 1973).

According to some studies, the use of certain herbicides at normal doses has no consequence either on the nitrifying bacteria or on nitrification. To this group belong simazine, prometryn, neburon and fluometuron (Horowitz et al., 1974) and dalapon and paraquat (Namdeo and Dube, 1973). Some studies report depressive results of herbicides on nitrification including atrazine (Voets et al., 1974) and prometryn, metabuzin, medipham, goltrix and sencor (Simon-Sylvestre and Fournier, 1979). Other reports have demonstrated a stimulation of nitrifying bacteria by herbicides. Such effects have been observed with simazine terbutryn and bentazone (Edwards, 1989) and prometryn (Simon-Sylvestre and Fournier, 1979).

Fungicides may severely suppress nitrification in soil. To this group belong captan (9 kg ha^{-1}), thiram (6.7 kg ha^{-1}), dicloran (2 kg ha^{-1}) and formalin at 500 litres ha^{-1} (Wainwright and Pugh, 1974), lanstan (1 kg ha^{-1}) and dichloran at 1 - 10 kg ha^{-1} (Caseley and Broadbent, 1968) and anilizine and maneb at 1.5 to 96 kg

ha^{-1} (Dubey and Rodriguez, 1970). Maneb, zineb and tribasic copper applied repeatedly to the soils also decreased nitrification and nitrogen mineralization (Dubey, 1970). Fungicides which are reported to have no effect on nitrification include benomyl at 1.0, 2.5 and 10 kg ha^{-1} (van Faassen, 1974) and ridomol (Golovleva and Finkel'Shtein, 1988).

Insecticides have an inhibitory influence on nitrification. These include carbofuran at 10 ppm (Palaniappan and Balasubramanian, 1986), lindane, malathion and baygon each at 10 ppm (Garretson and San Clemente, 1968), propoxur and aldicarb at the rate of 0.5 kg a.i. ha^{-1} (Kuseske et al., 1974) and fonofos, trichloronate and chlorpyrifos at 50 kg ha^{-1} (Shin-Chsiang Lin et al., 1972). Diazinon, chlorpyrifos, thionazin and trichloronate each at 1 or 10 kg ha^{-1} applied to a sandy loam, either had no effect or slightly inhibited the nitrification process (Tu, 1970). Counter at rates of 0.1 to 10 kg ha^{-1} in three soil types had no effect on nitrification (Laveglia and Dahm, 1974).

In the present study it was found that the high levels of all three pesticides used significantly reduced the nitrification activity of both Darvel and Dreghorn soils (Tables 4.9 and 4.12). Ramkrishna and Sethunathan (1983) reported that carbaryl and its hydrolysis product 1-naphthol inhibited nitrification by a heterotrophic bacterium, a <u>Pseudomonas</u> at 50 μg/ml and by

chemoautotrophic bacteria, a Nitrosomonas and Nitrobacter, at 10 μg/ml, in pure culture. Carbaryl was also found to be detrimental to nitrification in sewage (Lieberman and Alexander, 1981). Three hydrolysis products of carbaryl, 2-chloro-1-naphthol, 4-chloro-1-naphthol and 1-naphthyl-N-chloro-N-methyl carbamate in basic medium showed inhibitory effects on bacterial nitrification (Poncin et al., 1982). But malathion applied at 50 mg kg^{-1} to a sandy loam soil in a laboratory experiment did not markedly affect the soil microflora (Ogunseitan and Odeyemi, 1985). From the Tables 4.9 and 4.12 it can be seen that malathion at low level did not affect significantly the nitrification activity of both Darvel and Dreghorn soils. Similar result was obtained by Nishio and Kusano (1978). They found that continuous soil treatment with malathion for 5 years did not affect the bacterial count and nitrification activity of soils at normal rate of application (1:1000 soln. 125 ml/m^2).

The effects of the pesticides on nitrification process in soil were found to be inhibitory and the inhibition was related to the pesticide concentration and varied with the type of soil.

4.3.3 GENERAL DISCUSSION

In this investigation of the effects of three pesticides on soil microbial processes, toxic effects were observed in all three cases. Such effects may be transitory, as observed by Tu (1989) for the effects of several insecticides, or more permanent. The pesticide may undergo transformations, such as microbial breakdown or adsorption, or a resistant microbial population may develop in the soil. In some cases when the products are chemically and biologically stable the effect remains constant throughout the test period. The effect can increase with time when a more harmful product is formed during degradation. The experiments conducted in this study do not permit a distinction between transitory and permanent effects as the experiments were carried out immediately after treatment of the soils with pesticide. It is expected that the effects observed in this study would be transitory and that there would be no long term effects on soil fertility and the ability of the soil to supply nutrients to plants by the breakdown of soil organic matter and mineralisation of plant nutrients. More permanent effects might be expected with repeated applications of pesticide over a growing season or over a number of years unless the soil population adapts to the pesticide when the effects would reduce with repeated exposure.

The effects of the pesticides on phosphatase activity were found to be greater in Darvel than in Dreghorn soil. Similar results were found for dehyrogenase activity in the two soils. In the case of nitrification differences between the soils are more difficult to determine because of the very rapid nitrification in Darvel soil. As noted on page 166 the results presented in Table 4.9 are considered to be the best measure of nitrification in Darvel soil and may be compared with the results for Dreghorn soil in Table 4.10. More significant effects were observed in Darvel soil than in Dreghorn soil. Both soils have similar pH but Darvel has a higher organic matter and clay content (Tables 2.1 and 2.2). It is therefore surprising that all three measures of soil microbial activity showed a greater effect of the pesticides on Darvel soil.

Dehydogenase activity was more sensitive to the effects of pesticides than either phosphatase activity or nitrification. Dehydrogenase is a general measure of respiration and organic matter breakdown and is expected to reflect inhibition of any part of the microbial population. In particular captafol would be expected to have a large effect due to its fungicidal activity. Phosphatase activity is partly due to cellular and partly due to soil bound enzymes. Soil bound enzymes may be expected to be less affected by pesticide addition than enzymes within cells. There were decreases in activity in

the nitrification experiment, but due to the greater variability between replicates these were statistically less significant. Both captafol and malathion resulted in large decreases in nitrification.

In a study of the effects of organophosphorus and carbamate insecticides on phosphatase activity in soil, Hong and Kim (1986) found the inhibitory effect decreased in the order dithiophosphate > thiophosphate > phosphate > carbamate. Such a trend was not observed between malathion (dithiophosphate) and carbaryl (carbamate) in the present study.

4.3.4 FURTHER INVESTIGATIONS

In any thesis there is always some work that cannot be fully completed. Unexplained differences were observed between the controls in enzyme assays run in different batches for both phosphatase and dehydrogenase measurements. This could be studied by carefully testing the possible reasons discussed in Section 4.3.1.

In order to find out if the effects of the pesticides are transient or persistent, the changes in activity could be monitored over a longer period taking samples at intervals after treatment with pesticide. The treated samples could be incubated under various test conditions favouring microbial activity and recovery of the population.

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