# STUDIES ON THE MEASUREMENT AND BEHAVIOUR OF NITROGEN IN SOIL

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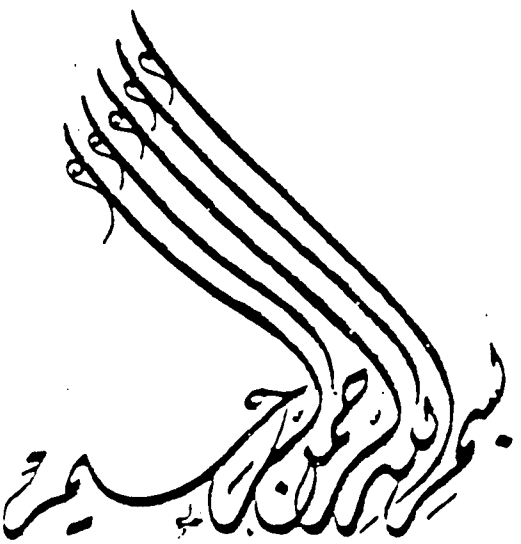

In the Name of ALLAH

"Most Gracious, Most Merciful"

"He Who taught (the use of) the Pen,"

"Taught man that which he Knew not."

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CONTENTS

## SUMMARY

This study of the measurement and behaviour of nitrogen in soil is split into three main parts: 1) An investigation of the Kjeldahl digestion method for measuring total nitrogen in soil and plant materials. 2) A study of the effect of air drying, temperature and repeated ammonium application on nitrification rates and 3) An investigation of ammonium contamination at a former nylon factory site.

The investigation of the Kjeldahl digestion method was made by comparing measured values of total nitrogen using a standard Kjeldahl digestion method and a salicylic acid modification digestion method. For each method three different catalyst mixtures were used. Measurements were made of certified reference plant materials (hay and cabbage) and a comparison was made of two soil samples.

The standard Kjeldahl digestion method with 1 g of sodium sulphate/copper sulphate mixture (100:10) measured significantly lower nitrogen ($P<5\%$) than the certified reference value for hay but not cabbage. Significantly lower ($P<5\%$) total nitrogen was measured in soil samples than with 2.5 g of sodium sulphate/copper sulphate mixture (100:10) and Kjeltabs (2.5 g potassium sulphate/copper sulphate/selenium, 100:10:1). The reason for the lower recovery of total nitrogen with 1 g of catalyst is the lower digestion temperature which causes incomplete digestion. The 2.5 g of catalyst mixture and Kjeltabs with the standard method gave significantly ($P<5\%$) higher total

nitrogen than the certified values for both hay and cabbage. The high values of total nitrogen measured for plant material were probably due to variable recovery of the high levels of nitrate which were present in the plant material at 3.1 mg $g^{-1}$ (hay) and 3.2 mg $g^{-1}$ (cabbage).

The salicylic acid modification method measured significantly higher total nitrogen than the certified reference values using all catalyst mixtures with both plant materials. This higher recovery of total nitrogen was due to partial recovery of nitrate as the method used for the certified values would not have recovered nitrate. Nitrate recovery was however, incomplete (35-75%) by the salicylic acid modification method. The salicylic acid modification method recovered significantly lower total nitrogen from soil samples than the standard Kjeldahl method. The soil samples contained insignificant levels of nitrate and the salicylic acid modification method results show nitrogen losses particularly in the treatment using Kjeltabs which contain selenium.

The difference between the steam distillation method and the Technicon Autoanalyser method of ammonium determination was nonsignificant. No interference effect of selenium or copper was found when using colorimetric analysis for ammonium nitrogen.

The possible use of Kjeldahl digests for multielement analysis was investigated by analysing for phosphorus and potassium in the digests of certified reference materials. The phosphorus recovery for plant material showed little effect of catalyst mixture on methods of digestion. The

measured values were significantly greater (P<5%) than certified values for both hay and cabbage. The potassium recovery was also significantly higher (P<5%) than the certified values for the plant materials. The potassium recovery for both plant materials was effected both by the method of digestion and catalyst mixture. These digestion methods were not considered suitable for multielement analysis.

The Ascorbic acid/Molybdate method and Metavanadate/ Molybdate method for phosphorus determination were significantly different (P<5%). The Ascorbic acid/Molybdate method significantly measured lower phosphorus than the Metavanadate/ Molybdate method. No interference effect of selenium or copper was found for colorimetric analysis of phosphorus.

A laboratory incubation experiment was carried out to study the effects of: air drying of soil samples, incubation temperatures of 0, 5, 10, 15, and 20 °C and two applications of ammonium, on the nitrification rate in five soils. The nitrification rates were significantly slower (P<5%) in air dried soils than fresh soils. The air dried soils also showed a clear lag period before nitrification started. The mineralisation rates were significantly greater (P<5%) in air dried soils than in fresh soils. Both nitrification rates and mineralisation rates decreased with decreasing temperature in all soils. Sharp decreases in nitrification rates were observed below 10 °C. The effect of temperature on nitrification rates and mineralisation rates showed a good fit to a straight line Arrhenius

relationship. The B coefficients (a constant related to temperature sensitivity) for ammonium disappearance and nitrate formation were similar in all soils. Values for the B coefficients ranged from -8255 to -10246 °K. These values are slightly higher than other published values. Nitrification of ammonium sulphate caused a decrease in pH. The application of second dose of ammonium sulphate did not significantly effect nitrification rates in four out of five soils tested. In the Darvel soil a significant decrease ($P<5\%$) in rate was probably due to the fall in pH which occurred from 5.8 to 5.2 in first incubation period and from 5.2 to 4.8 in the second.

An investigation of ammonium contamination at a former nylon factory site where ammonium leaching was contaminating an adjacent stream was carried out by conducting four surveys for collecting soil and water samples at different depths down to 8 metres. The soil and water samples were analysed for ammonium, pH, organic matter content, conductivity, soil particle size distribution and cation exchange capacity. The ammonium analysis showed that contamination was localised to the area of the site where ammonia was delivered and utilised during the factory operation period. The ammonium level increased with depth below the water table and in some boreholes a peak of ammonium was found at 3 metres to 7 metres depth indicating that ammonium may be present as a layer. The maximum level of ammonium contamination measured was up to 496 mg N $kg^{-1}$ in the soil samples and 519 mg N $l^{-1}$ in the ground water samples. The subsoil is very sandy,

low in organic matter content and clay, therefore it has a low cation exchange capacity (10.7 $mmol_e$ $kg^{-1}$). The exchangeable ammonium was a significant proportion of the exchange sites and approximately 25 % of the ammonium was free in the pore solution. In view of this it is surprising for ammonium to be present 14 years after closure of the factory. It is possible that the ammonia contaminated ground water is trapped in a bowl shaped depression in the boulder clay underlying the sand strata. It must be expected that ammonium will continue to leach for many years.

# CHAPTER 1

# INTRODUCTION

## 1.1 BACKGROUND

Nitrogen is a very complex element in its behaviour in the environment. It occurs in the four spheres of the environment, namely the lithosphere, the atmosphere, the hydrosphere and the biosphere. Table 1.1 shows an approximate inventory of nitrogen in the four spheres. The bulk of the nitrogen (about 98 %) exists in the lithosphere, and most of the remainder in the atmosphere. The lithospheric nitrogen is the sum of the nitrogen in the earth's sedimentary, metamorphic and igneous rocks as silicate minerals, coal, sea bottom organic compounds and surface soils. These constituents of the lithospheric nitrogen are the major fraction of the environmental nitrogen by overall occurrence. In the hydrosphere nitrogen occurs as molecular $N_2$, ammonium, nitrite, nitrate and as dissolved and particulate organic matter in the oceans, rivers, lakes and underground water (Stevenson, 1982). The inert gas $N_2$ in the atmosphere and the organic forms of nitrogen in the biomass are the largest fractions of these two spheres. However, the concentration of nitrogen (the weight of nitrogen per 100 g of substance) is highest at a concentration of nitrogen of 78 % in the atmosphere, followed by 3.1 % in the biosphere and 0.002 % in the lithosphere and the hydrosphere. The nitrogen content of soil ranges from less than 0.02 percent in subsoils to 2.5 percent in peats. The surface layer of most cultivated

soils contains between 0.06 and 0.5 percent N. (Bremner and Mulvaney, 1982).

Table 1.1 Selected data on nitrogen levels in the four spheres of the environment (Stevenson, 1982).

| Sphere | Terrogram of N |
|---|---|
| Lithosphere | $1.636 * 10^{11}$ |
| Igneous rocks of the mantle | $1.620 * 10^{11}$ |
| Terrestrial soils | |
| Organic matter | $2.200 * 10^{5}$ |
| Clay fixed | $2.000 * 10^{4}$ |
| Atmosphere | $3.860 * 10^{9}$ |
| Hydrosphere | $2.300 * 10^{7}$ |
| Biosphere | $2.800 * 10^{5}$ |

The significance of nitrogen arises from the fact that the air over each square metre of the earth's surface contains about 900 kg of nitrogen and that after Carbon, Hydrogen and Oxygen, no other element is so intimately associated with the reactions carried out by living organisms.

Many difficulties are encountered in determining the distribution of nitrogen and the reactions carried out by living organisms ( biosphere ) in soil. Unlike the other spheres, the soil nitrogen is in a constant state of flux. Also, the matter of the surface soil is not uniformly distributed, and the nitrogen contents and transformations of nitrogen by different organisms vary widely in soils.

## 1.2 SOIL NITROGEN CYCLE

Figure 1.1 shows the main chemical and biological processes of nitrogen transformations in the soil and the direction in which changes may occur. Atmospheric nitrogen is added to soil by several mechanisms under natural conditions. Electrical discharges in the atmosphere, such as lightning, cause some slight combination of oxygen and nitrogen and this leads to the passage of nitrogen into the soil as nitrates dissolved in rain water. Nitrogen compounds present in the atmosphere return to soil with rainfall. Nitrogen fixing microorganisms, living free in soil or in symbiotic associations with plants, are an important source of nitrogen by converting atmospheric nitrogen into nitrogen compounds which pass into the soil. In addition, nitrogenous fertilisers such as urea, ammonium sulphate and ammonium nitrate and nitrogen based compounds are prepared by industrial fixation of atmospheric nitrogen and are then applied to soil. Soils also receive nitrogenous compounds from manures, organic waste and due to the death and decay of plants and animals. These nitrogen compounds, either organic or inorganic, in soil are utilised by the soil micro-organisms and plants.

The soil micro-organisms are fundamentally involved in important changes to the nitrogen compounds in soil. A wide range of heterotrophic micro-organisms, regardless of whether they are bacteria, fungi or protozoa, need organic material as a source of combined carbon from which they can derive energy by respiration and carbon for

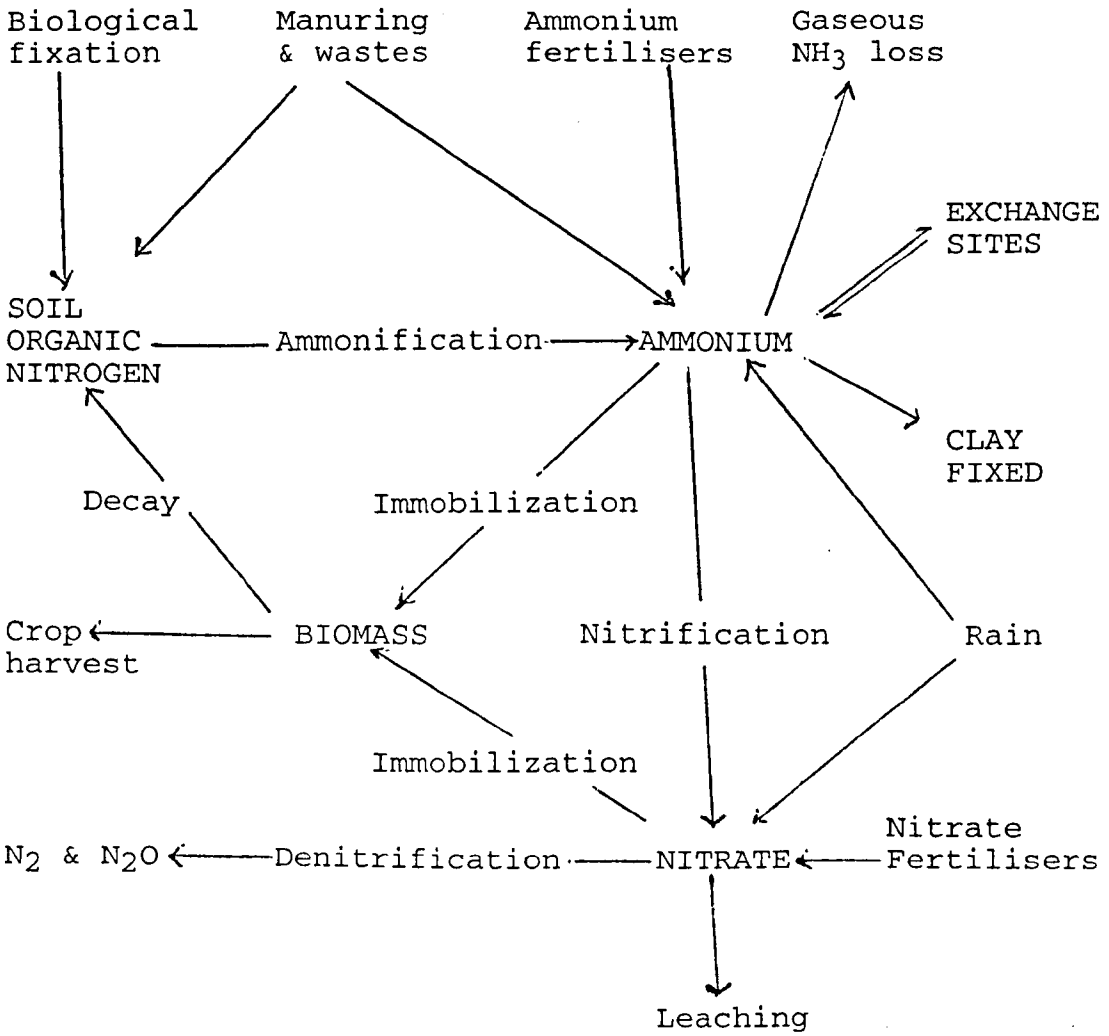



Figure 1.1 The Soil Nitrogen cycle.

cell synthesis. To accompany this carbon they also require nitrogen and other nutrients such as phosphorus, potassium, calcium and sulphur etc. As protein is progressively decomposed, ammonium is the endpoint of degradation and this will be used by the micro-organisms for cell synthesis (Wild, 1988). If more nitrogen is present in the organic substrate than is required by the feeding micro-organisms the surplus is released as a waste product as ammonium-N. The conversion of organic nitrogen to the more mobile, inorganic state is known as nitrogen mineralisation. If insufficient nitrogen is present in the substrate the micro-organisms will draw on the mineral nitrogen in the soil to make good the deficit and nitrogen immobilization will result.

As a consequence of mineralisation, ammonium is generated and organic nitrogen disappears. The formation of ammonium from the organic matter by micro-organisms is called ammonification. It provides readily available ammonium nitrogen for cereals and grassland species and could be regarded as the preferred nitrogen supply as it is less readily lost from the soil than nitrate and is used more efficiently within the plant. The ammonification process also provides ammonium for nitrification. Nitrification is usually associated with the energy-yielding reactions in the metabolism of autotrophic bacteria. The ammonium nitrogen is first converted into nitrite by Nitrosomonas and then to nitrate by Nitrobacter. The term nitrification is usually used for these two stages of the oxidation of ammonium into nitrate.

In addition to these nitrogen transformation processes, soil micro-organisms are also involved in denitrification. Under anaerobic conditions, nitrate may be readily used by facultative anaerobic denitrifying bacteria, resulting in the formation of gaseous products like $N_2$ and $N_2O$ which are lost into the atmosphere.

Not all transformations of nitrogen in soil are mediated by micro-organisms. Through the physicochemical association of humic material with mineral matter, metallo-organic and organo-clay complexes are formed, whereby the organic nitrogen compounds are protected against attack by micro-organisms. The positively charged ammonium ion undergoes substitution reactions with other cations of the exchange complex and can also be fixed by clay minerals. Nitrate in contrast to ammonia is not adsorbed by the soil colloids and, therefore, it is easily leached when precipitation exceeds evaporation.

The rate of microbial activity in soil depends on several factors, including soil moisture content, temperature, availability of oxygen, pH and substrate availability. Organic nitrogen is mineralized at moderate or at excessively high moisture levels. Drying and wetting cycles may affect the nitrogen mineralisation rate by making inaccessible substrates more easily available to microbial action. Drying may cause cell disintegration. Laboratory experiments with soil have shown that alternate drying and rewetting cycles result in an extra release of carbon dioxide, and frequently an extra production of mineral nitrogen ( Birch, 1958 and Bottner, 1985 ). Khan (1987)

reported that incubation of fresh soil samples seems to be more reasonable than incubation of air dried soil samples for mineralisation studies.

Temperature may affect the mineralisation sequence, as each biochemical step is catalysed by temperature sensitive enzymes produced by microorganisms whose growth is in turn conditioned by temperature. Thus, at 2 °C, the microflora slowly mineralize the organic complexes, but there is no ammonium or nitrate formed when soil is frozen. Nitrification is markedly affected by temperature, and many investigations have confirmed the fact that below 5 °C and above 40 °C the rate is very slow (Alexander, 1977 ).

Mineralisation is influenced by the pH of the soil. All other factors being equal, the production of inorganic nitrogen ( ammonium plus nitrate ) is greater in neutral than acid environments ( Ishaque and Cornfield, 1972 ). In acid conditions nitrification proceeds slowly, even in the presence of an adequate supply of ammonium, and the responsible species are rare or totally absent at low pH. Although an exact limiting pH cannot be ascertained, the rate falls off markedly below pH 6.0 and becomes negligible below 5.0 ( Dancer et al., 1973 ).

It has been reported that addition of nitrogen fertilizer sometimes enhances the mineralisation rate of native organic nitrogen in soils (Wickamasinghe et al., 1985). On the other hand, such amendments appear to have no influence or an adverse effect on the mineralisation process in other soils. For example, Williams (1975) found that addition of nitrogen (50 mg N $kg^{-1}$ soil) as ammonium

sulphate decreased the quantity of mineralizable nitrogen in incubated coal mine soils. He suggested that this may be due to the increase in acidity that results from the addition of ammonium sulphate. Under ordinary conditions the rate of nitrogen mineralisation is closely correlated with the total nitrogen content, and therefore soils rich in nitrogen liberate more inorganic nitrogen than those deficient in total nitrogen in a given time interval.

## 1.3 SOIL BIOCHEMISTRY OF NITRIFICATION

A major process of the soil nitrogen cycle is the oxidation of inorganic nitrogen in the form of ammonium into nitrate. The conversion of the ammonium into nitrate involves two reactions used by only a few genera of autotrophic bacteria to generate energy. The most common are Nitrosomonas and Nitrobacter. The energy yield for the reaction is low, resulting in very slow growth rates. It can be represented by the following overall reaction:

$NH_4^+$ ------> $NO_2^-$ ------> $NO_3^-$

Ammonium oxidation is restricted to five genera of bacteria, the most common being Nitrosomonas sp. The energy yield is only 272 kJ $mole^{-1}$ of $NH_4+$ oxidised compared to the oxidation of glucose which yields 2872 kJ $mole^{-1}$. Hydroxylamine is an intermediate in the reaction. The reaction generates acidity as protons are released:

$NH_4^+ + {}^3/_2O_2$ ------> $NO_2^- + H_2O + 2H^+$

Ammonium contains nitrogen in its most reduced state, but it is not readily oxidised. The direct oxidation of ammonium to hydroxylamine by oxygen is endergonic, and therefore requires a special type of enzyme or chemically reactive species. Once the first N-O bond is formed the subsequent oxidation is more favourable which allows ammonium oxidation to nitrite to be used as an energy source (Wood, 1989).

In the autotrophic nitrifying bacteria hydroxylamine is formed by ammonium monooxygenase (a copper-containing enzyme):

$NH_4^+ + O_2 + H^+ + 2e^-$ ------> $NH_2OH + H_2O$

The reaction is a reduction and relies upon electrons produced by the subsequent oxidation of hydroxylamine:

$NH_2OH + H_2O \text{ ------> } NO_2^- + 5H^+ + 4e^-$

A small portion of the electrons transported across the membrane by this reaction are transported back to generate reductants to allow the conversion of ammonium to hydroxylamine. This is an unusual reaction because the cell relies upon the monooxygenase to produce hydroxylamine which is the electron donor for the reaction.

Nitrite oxidation, which is even less energetically favourable (71 kJ $mole^{-1}$ of nitrite ), is restricted to one genus of bacteria, Nitrobacter:

$NO_2^- + {}^1/_2O_2 \text{ ------> } NO_3^-$

Reverse electron transport of approximately one-fiftieth of the electrons produced from nitrite is used to generate reductant (NADH) which may be used, for example, in the assimilation of carbon dioxide (Wood, 1989).

## 1.4 NITRATE LEACHING

The nitrate formed at the completion of the mineralisation and nitrification processes is leached down from the soil when soil moisture is at field capacity or more. The climatic conditions of Britain can be illustrated by looking at data for two areas of the country. The temperature, rainfall and potential evapotranspiration data for two areas of England from agricultural climatic statistics are given in Tables 1.2 and 1.3 (MAFF, 1976).

Table 1.2 Climatic data of North West England ( Cumbria ) (MAFF, 1976).

| Month | Temperature air ... | earth °C.. | Rain mm | PT mm | Day length hrs |
|---|---|---|---|---|---|
| JAN | 2.8 | 3.1 | 97 | 1 | 9.3 |
| FEB | 3.0 | 3.1 | 70 | 8 | 10.9 |
| MAR | 4.8 | 4.2 | 61 | 29 | 13.1 |
| APR | 7.4 | 7.2 | 65 | 47 | 15.3 |
| MAY | 10.3 | 11.0 | 68 | 75 | 17.7 |
| JUN | 13.1 | 14.5 | 69 | 81 | 19.2 |
| JUL | 14.4 | 16.0 | 83 | 80 | 18.3 |
| AUG | 14.3 | 15.8 | 106 | 65 | 16.3 |
| SEPT | 12.7 | 13.6 | 109 | 37 | 13.9 |
| OCT | 9.8 | 10.7 | 105 | 19 | 11.7 |
| NOV | 5.9 | 6.8 | 105 | 3 | 9.8 |
| DEC | 3.9 | 4.4 | 107 | -1 | 8.8 |
| TOTAL | | | 1045 | 444 | |

Growing season: 237 days Apr 3- Nov 26
Potential Transpiration: 403 mm
Grazing season: 150 days Apr 10- SEPT 7
Degree-days above 10 °C May to Oct: 550
Winter degree-days below 0 °C: 160
Effective Transpiration : 370 mm
Grass Drought factor : under 5 days
Mean last frost : Mid - May
Maximum Summer Soil Moisture Deficit 52 mm
Return to capacity SEPT 7
Excess Winter Rain 550 mm
End of capacity May 9

Table 1.3 Climatic data of Cambridge area. (MAFF, 1976).

| Month | Temperature air ... | earth °C.. | Rain mm | PT mm | Day length hrs |
|---|---|---|---|---|---|
| JAN | 3.0 | 3.6 | 48 | 1 | 9.6 |
| FEB | 3.4 | 3.6 | 38 | 10 | 11.1 |
| MAR | 5.7 | 5.2 | 38 | 33 | 13.0 |
| APR | 8.4 | 8.5 | 37 | 57 | 15.1 |
| MAY | 11.5 | 12.2 | 44 | 83 | 17.2 |
| JUN | 14.6 | 15.4 | 47 | 94 | 18.4 |
| JUL | 16.5 | 17.1 | 56 | 94 | 17.8 |
| AUG | 16.1 | 16.8 | 60 | 76 | 15.9 |
| SEPT | 14.2 | 15.1 | 49 | 48 | 13.8 |
| OCT | 10.7 | 11.6 | 49 | 22 | 11.8 |
| NOV | 6.4 | 7.4 | 58 | 5 | 10.1 |
| DEC | 4.0 | 4.8 | 50 | 0 | 9.1 |
| TOTAL | | | 574 | 523 | |

Growing season: 249 days Mar 26- Nov 30
Potential Transpiration: 485 mm
Grazing season: 254 days Mar 31- Dec 10
Degree-days above 10 °C May to Oct: 815
Winter degree-days below 0 °C: 145
Effective Transpiration : 338 mm
Grass Drought factor : under 50 days
Mean last frost : Late April
Maximum Summer Soil Moisture Deficit 113 mm
Return to capacity Dec 10
Excess Winter Rain 130 mm
End of capacity Mar 27

The data in Tables 1.2 and 1.3 show that rainfall in England and Wales is fairly evenly divided throughout the year. The north western area of England is wetter than central England due to higher rainfall, less potential evapotranspiration and lower temperature. The soils of the northern area show a soil moisture deficit for a shorter period of May to September, while the soils of central England show a soil moisture deficit from March to

December. The climatic conditions of the northern area are therefore more favourable for leaching and denitrification of nitrate nitrogen from September to May. Late summer is still warm and the soil is returning to field capacity thus this period favours nitrification and mineralisation. The climatic conditions of central England provide favourable conditions from December to March for nitrate leaching. During autumn, mineralisation and nitrification rates are critical. As the temperature decreases the soil conditions also become wetter. The micro-organisms experience favourable conditions for mineralisation and nitrification. The nitrates formed during this period are subject to leaching in early winter when the soil reaches field capacity.

To minimise the nitrate leaching in Britain the Ministry of Agriculture, Fisheries and Food, code of good agricultural practice for the protection of water recommends that nitrogen fertiliser should not be applied between 1 September and 1 February unless the crop needs it during this time. Nitrogen fertilisers applied during this period cause nitrate pollution in underground water. (MAFF, 1991). However, plentiful supplies of good quality grass are vitally important on all livestock farms. The key factor on these farms is getting grass growth as early in the season as possible. The right choice of fertiliser and the date of the first application of fertiliser have an important role to play in ensuring this. The farmers are also in need of information on the accurate amount of fertilisers needed to grow early grass for ample supplies

of good quality forage. In addition to reduced concentrate bills, heavier liveweight gains and better mineral content of spring grass have also been given as important reasons why this grazing is highly valued. In early spring, applications of ammonium fertilizer are under risk of losses by run off and leaching if nitrification occurs.

For the last fifteen years the Farmers Weekly magazine has used the T sum 200 system to recommend the date of first application of nitrogen fertiliser. The T sum is measured by adding together all the positive average daily temperatures from January 1, until the sum of 200 °C is reached. At this point fertilisers are applied. Maps are published weekly showing which areas of the country have reached T sum 200.

The philosophy behind fertilising at T sum 200 is simple. When applying fertiliser earlier, farmers run the risk of losing nutrients through leaching. Applying it too late, leads to valuable lost growing days. Applying at, or around, T sum 200 minimises these risks. Thus the use of the T sum reduces the problem of time of application but not the accurate amount of the fertiliser dose.

Farmers growing winter crops have similar problems of timing early nitrogen applications. After harvesting of cereal crops in August/September there may be residual nitrogen in the profile and conditions could be favourable for mineralisation and nitrification. If the next crop is a spring sown crop such as spring cereal, potato, or sugarbeet, then the soil is not covered with a crop for up to six or more months.

In Pakistan due to the dry and warm climate pollution by nitrate and nitrate leaching is not a problem. However, the soils of Pakistan are deficient in plant nutrients due to the low organic matter content and fertilisers are a major source of plant nutrients for high crop yield. About 70 % of the cultivated area is canal irrigated and 30 % is rainfed. The research organisations and extension workers in irrigated areas recommend two fertiliser doses, one at the time of sowing and the second at first watering. Due to the high cost of fertilisers farmers apply less fertilisers than the recommended dosage. The farmers of the rainfed area grow crops which require low amounts of fertiliser. The farmers are always unsure of the amount of mineralized and nitrified nitrogen which will be produced in the growing season or during the fallow period. If more were known about the mineralised and nitrified nitrogen more effective use could be made of nitrogen fertilisers.

The principles set forth with regard to the metabolism of nitrogen can frequently be extended to the transformations of several elements, and consideration of the various steps of nitrogen cycle will lead to some understanding of the microbiologically induced changes of other major nutrient elements such as phosphorus and sulphur.

## 1.5 AMMONIUM POLLUTION

Soil is one of the most important of our natural recourses. It supports and provides nutrients for the plants and animals that provide our food, fibre and shelter. It is also the receptacle for much of our waste material, helping to correct and often hide many of our mistakes and oversights. If managed properly, the soil provides protection for our environment.

The soil is also non-renewable for all practical purposes, highly variable, and complex, especially from a chemical and biological stand-point. It is essential that we have the best possible understanding of the nature and properties of our soils if we are to make the most efficient use of them for food and fibre production, and at the same time preserve them for future generations.

The demands on soil for cultivation, housing and business is increasing with increase in population. The efforts of scientists, agricultural chemists, farmers and planners have been diverted to improve waste, contaminated and polluted land, as well as improve the environment from contamination or pollution caused by human activities.

The subject of contaminated soils has become one of the most important issues in the world from the environmental point of view. It is very difficult to pin-point an exact definition, suitable for universal application, of contaminated land due to the wide range of interests involved. Some land contains high natural levels of elements and compounds which may be regarded as

contaminants in a general sense, but definitions usually relate to contamination as a result of human activity. Land which contains substances which, when present in sufficient quantities or concentrations, are likely to cause harm, directly or indirectly, to man, the environment, or on occasion to other targets are called contaminated. There is a difference between contaminated and derelict lands. It is therefore useful to clarify the differences between contaminated and derelict land. Although the focus has been on contaminated land, there are links between it and derelict land, and the two often go hand in hand. Derelict land can be defined as land so damaged by industrial or other development it is incapable of beneficial use without treatment. Contaminated land is not exclusively derelict land however, because a site can be contaminated and yet still be fully operational. Equally, derelict land is not necessarily contaminated. The water quality standard authorities define contaminated land as land that breaches water quality or where there is evidence of poor water quality of surface or ground water.

Over the last few years the subject of nitrogen contamination in the form of nitrate in soil and water has become one of the most debated environmental issues in the United Kingdom. Pollution by ammonium in agricultural land is rare. However, some land which was previously used for industry may be contaminated during its operation from spillage of materials, or from the open-air storage of solid raw material or products. For example problems in the redevelopment of gasworks and similar sites have been

observed in the form of ammonium contamination. These coal carbonisation sites are usually contaminated with coal particles, coke, treated oxides, sulphur, lime, acids, bases, catalysts, and heavy metals as well as ammonium. As a result, there is widespread interest in how the problem may be dealt with technically, politically, legally and financially. In this environmental issue the National Rivers Authority in England and Wales and the seven River Purification Boards in Scotland are responsible for monitoring and enforcing water quality standard.

## 1.6 OBJECTIVES OF STUDY

This thesis covers studies of methods of Kjeldahl digestion for the determination of total nitrogen in plant and soil samples, measurements of nitrification and mineralisation in soil as affected by climatic factors and an investigation of a case of ammonium nitrogen contamination at a former industrial site.

The thesis has been divided in six chapters. The review of literature related to each topic is covered in its relevant chapter. A description of each chapter is given below:-

Chapter one provides a general introduction and sets out the aims of the work to be undertaken, and the objective of each chapter.

Chapter two describes the routine methods, apparatus, procedures, preparation of reagents and standard solutions, setting up of apparatus, and calculations of results followed during this study.

Chapter three deals with the effect of catalyst mixture and Kjeldahl digestion methods on the total nitrogen determination in certified reference plant material and in soil samples. The comparison of total nitrogen, phosphorus and potassium recovered from certified reference materials was made with the respective certified values. In addition comparisons were made of total nitrogen determination by the steam distillation method versus the Technicon Autoanalyzer method and of phosphorus measured by the Ascorbic/molybdate method and the Metavanadate/ molybdate method were made.

Chapter four is devoted to incubation studies under controlled laboratory conditions to measure the effects of air drying and temperature on the nitrification of applied ammonium sulphate in a range of soils.

Chapter five describes several investigational surveys carried out to investigate the source of ammonium pollution in a stream adjacent to a former nylon factory area.

Finally Chapter six provides a general discussion of these studies of the measurement and behaviour of nitrogen in soil.

# CHAPTER 2

# METHODS AND MATERIALS

This chapter is intended to provide a brief introduction, and describe the apparatus, reagents and preparation of reagent solutions, analytical procedures for determining concentrations in solution, and calculation of concentrations in the materials analysed by the various analytical methods which were used in the present study.

## 2.1 WASHING OF GLASSWARE AND PLASTICWARE

All glassware and plasticware was selected to ensure it was free from chips and scratches. Prior to washing, all ink marks and previous labels were removed with ethanol. It was then washed with hot tap water three times and soaked overnight in a 2 % solution of Decon 90. The materials were then thoroughly rinsed five times with hot water, twice rinsed with deionized water, and finally dried in an oven. Glassware was dried in a 110 °C oven while plastic material was dried in a 70 °C oven. ( Mazumder, 1990.)

## 2.2 DETERMINATION OF AMMONIUM NITROGEN

Ammonium nitrogen in sample solutions was measured by two methods: Steam distillation and Technicon Auto-Analyser.

### 2.2.1 Steam Distillation Method for Ammonium.

This is a manual method of determining ammonium nitrogen by liberating ammonia with the flow of steam from

an alkaline solution and trapping it in boric acid indicator mixture solution. The quantity of ammonium is estimated by titrating the indicator mixture with acid.

2.2.1.1 Reagents

Sodium hydroxide 10 M :- Working in a fume cupboard 40 g of NaOH tablets were dissolved in 80 ml of deionized water in a plastic bottle. After cooling to room temperature, the solution was made up to 100 ml.

Tris hydroxymethyl amino methane (THAM) 0.01 M :- The THAM buffer was prepared by dissolving 12.114 g of THAM in 100 ml of water to give 1 M THAM and 1 ml of this THAM solution was diluted to 100 ml to give 0.01 M THAM.

0.01 M HCl :- 10 ml of M HCl was diluted with deionized water in a 1000 ml volumetric flask and made up to the mark. The 0.01 M HCl was standardized by titration with 0.01 M THAM.

Boric acid indicator mixture:-

0.5 ml of 0.5 % methylene blue solution, 3.75 ml of 0.1 % methyl red solution and 1.25 ml of 0.1 M NaOH solution were added to 250 ml of 2 % Boric acid solution. The volume of NaOH used was found by trial and error based on the formation of a green colour when the indicator was diluted with water. The volumes of the two indicators were chosen to give a sharp end point when the indicator mixture

was titrated against 0.01 M HCl. Minor adjustments were made to improve the sharpness of the end point if required.

2.2.1.2 Apparatus

This was a modification of the apparatus described by Bremner and Breitenbeck (1983). Steam was supplied from a 5 litre round bottom glass flask containing 4 litres of deionized water and boiling chips ( to promote smooth boiling ). The heating was controlled to give a steam flow rate of 5-6 ml of distillate per minute. The steam passed through a drip trap and into the sample flask. The ammonia in the distillate then passed through a splash head into a condenser. The water flow in the condenser was kept such that the distillate temperature did not exceed 20 °C. The condenser was adjusted so that the tip of the condenser was just below the level of the boric acid indicator mixture in a 100 ml beaker.

2.2.1.3 Analytical Procedure

Before use the distillation apparatus was steamed out for 10-15 minutes to remove traces of ammonia in the system. An aliquot of 10 ml of sample solution was taken into a 50 ml distillation flask and 5 ml of 10 M NaOH was added. The flask was attached to the system immediately to avoid ammonia losses. 5 ml boric acid indicator mixture was placed under the condenser. Steam was allowed to pass through the apparatus by closing the stop cock on the steam by-pass of the distillation apparatus. When the distillate reached the 20 ml mark of the beaker, the beaker was

lowered so that the tip of the condenser was clear of the solution. When the distillate reached 35-40 ml, the steam by-pass was opened and the tip of the condenser was rinsed with water. The $NH_4^+$-N was determined by titration with 0.01 M HCL from a micro burette. The colour change at the end point was from green through grey then colourless, to the first permanent faint pink colour. Bremner and Edwards (1965) recommended magnetic stirring of the distillate while titrating. To perform a blank the same procedure was followed as described for the sample.

2.2.1.4 Calculation of N concentration

The concentration of N in the samples was calculated as follow:-

1 ml of 0.01 HCL = 140 mg N

Weight of N (µg) in sample aliquot. = ( sample titre - blank titre) * 140

$$\text{Concentration of N mg } l^{-1} = \frac{\text{weight of N (µg) in sample}}{\text{volume of sample aliquot (ml)}}$$

$$\text{Concentration in sample (mg kg}^{-1}\text{)} = \frac{\text{Concentration of N * volume of digest}}{\text{Weight of sample * Correction factor}}$$

2.2.2 Technicon AutoAnalyser Method for Ammonium.

Ammonium N was determined by a modification of the indophenol green method using a complexing reagent to prevent interferences due to the precipitation of hydroxides 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 mg $l^{-1}$ and with care 0-0.1 mg $l^{-1}$ (Brown, 1973). The Technicon AutoAnalyser method of ammonium was used for determining ammonium in Kjeldahl digests, water samples and soil extracts.

2.2.2.1 Preparation of reagents

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

Alkaline phenol

22.5 g of sodium hydroxide was dissolved in about 900 ml deionized water in a litre dark glass bottle. 50 g phenol was taken in a 1 litre beaker and approximately 500 ml of the 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 and mixed gently. The solution was degassed in an ultrasonic bath for 10 minutes.

Complexing reagent

50 g potassium sodium tartrate and 50 g sodium citrate were dissolved in approximately 900 ml water in a litre bottle. 1.2 g sodium nitroprusside was taken in a 100 ml beaker, 50 ml water was added to the beaker and stirred gently with a magnetic stirrer. The resulting solution was added to the citrate tartrate solution. 1 ml of 15 % Brij-35 was added and the volume was made to 1 litre. The solution was then mixed gently and degassed on an ultrasonic bath.

Sodium hypochlorite solution

50 ml sodium hypochlorite solution (10.14 % w/v available chlorine ) was diluted to 1 litre with deionized water and mixed thoroughly.

Ammonium nitrogen standard stock solution ( 1000 mg $l^{-1}$)

4.717 g of dried ammonium sulphate 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.

2.2.2.2 Apparatus

The Technicon AutoAnalyzer II was used for the analysis of ammonium-N, nitrite-N, nitrate-N and phosphate-P because of its sensitivity, speed and ease of use. The Technicon AutoAnalyzer II system consisted of a sampler, proportioning pump, a water bath at constant temperature

and colorimeter equipped with either 530 or 650 or 880 nm filters and phototubes. Results of the analyses 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. The reagent bottles were also put in a separate water bath at a constant temperature of 25 °C.

2.2.2.3 Procedure

The filtered solutions were analysed using the manifold shown in Figure 2.1 along with standard solutions, blanks and zeros. The samples were run at the rate of 40 per hour and the colour was developed in the water bath at 37 °C. The colour intensity was measured at 650 nm. The calibration graph for ammonium is linear from 0 to 5 mg $NH_4$-N $l^{-1}$. Samples with ammonium-nitrogen concentrations higher than 5 mg $l^{-1}$ were diluted into the range 0 to 5 mg $l^{-1}$ using an inbuilt diluter.

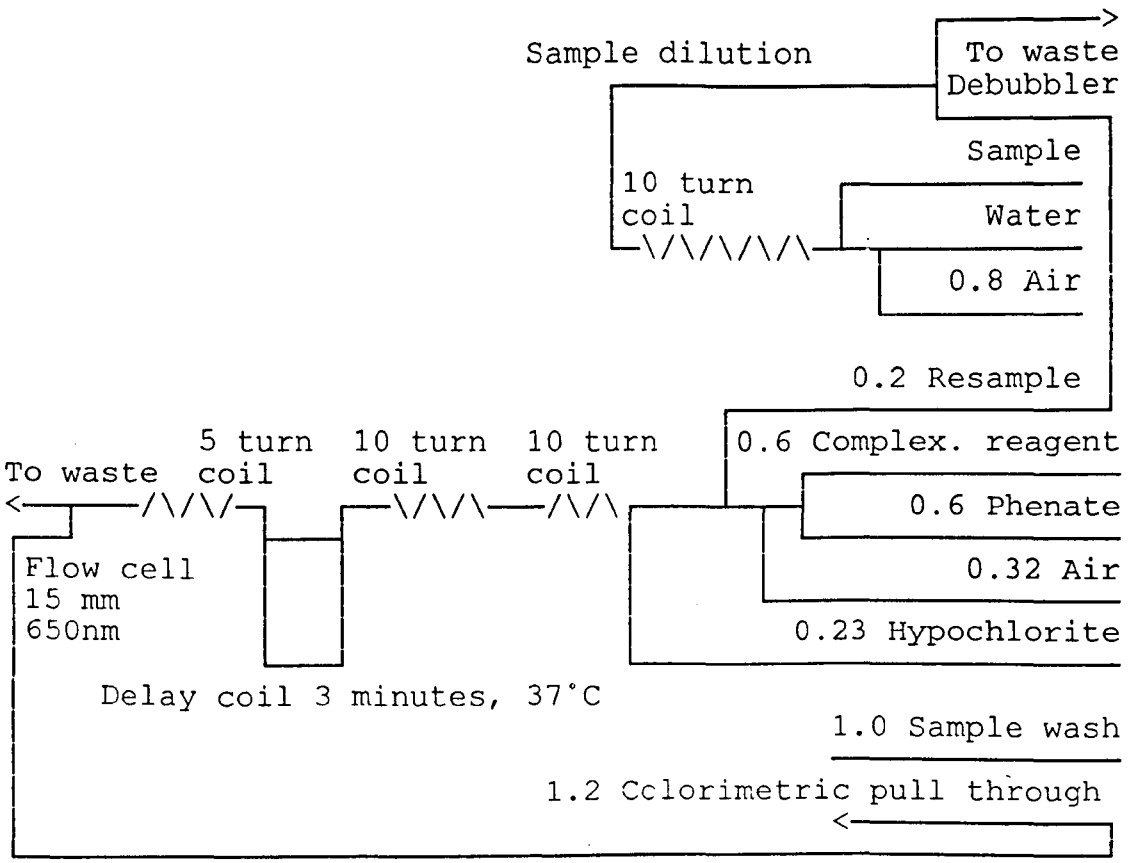



Figure 2.1 AutoAnalyzer Manifold For Determining $NH_4$-N

## 2.3 DETERMINATION OF NITRITE NITROGEN

In the automated system nitrite nitrogen was measured colorimetricaly by the Greiss reaction. Nitrite ions react with sulphanilamide by a diazotization reaction and the product couples with N-1-naphthylethylenediamine dihydrochloride to form a pink colour which was measured by colorimetry. The schematic diagram of the flow system for nitrite is shown in Fig 2.2 (Best, 1976).

### 2.3.1 Reagent preparation

All the reagent solutions were prepared with deionized water. After preparation the solutions were degassed in an ultrasonic water bath for ten minutes.

Buffer solution

Sodium tetraborate 22.5 g and 2.5 g sodium hydroxide were dissolved in 900 ml deionized water. The volume was made up to 1 litre in a stock bottle.

Greiss reagent

100 ml concentrated Hydrochloric acid was carefully added into approximately 800 ml deionized water and cooled to room temperature. 10 g sulphanilamide and 0.5 g N-1-naphthylethylenediamine dihydrochloride were dissolved in the cold acid solution and the volume was made up to 1 litre in a stock bottle.

Brij water 1 ml 15 % Brij-35 in a litre of water was also prepared and degassed.

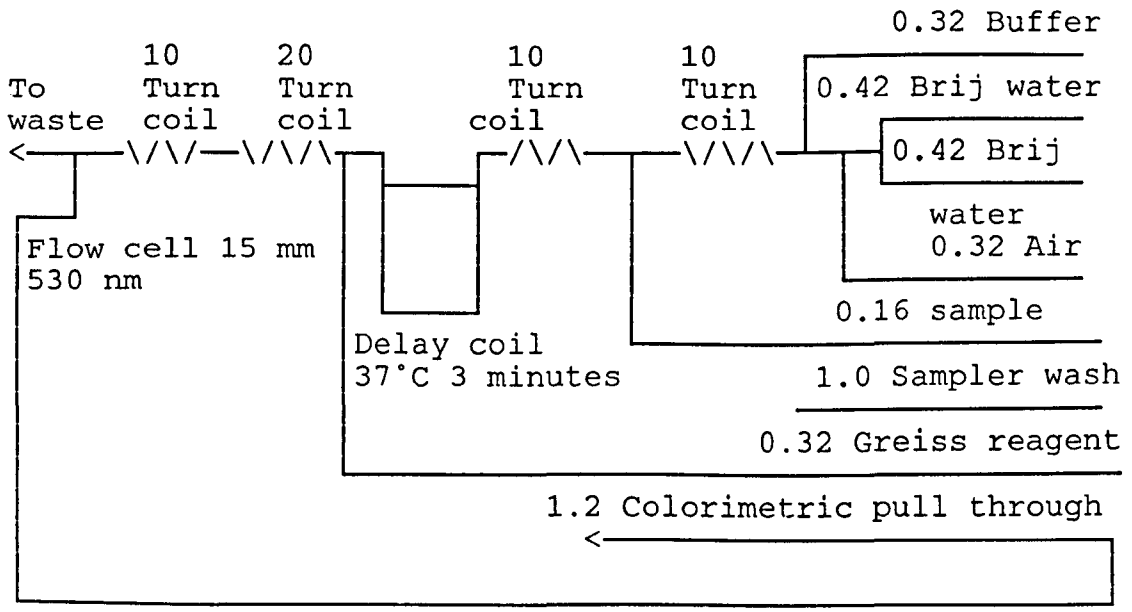



Figure 2.2 AutoAnalyzer Manifold For $NO_2$-N

Nitrite Nitrogen standard stock solution

4.926 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 solution.

2.3.2 Procedure

The manifold (Fig 2.2) was used for the determination of nitrite nitrogen in different extracting solutions. The samples were run at the rate of 50 per hour. The intensity of this colour was measured at 530 nm. Nitrite has a linear calibration in the range of 0-1 mg $l^{-1}$.

## 2.4 DETERMINATION OF NITRATE NITROGEN

In the automated system nitrate nitrogen was reduced to nitrite using hydrazine sulphate with a copper catalyst and the nitrite were determined using the Greiss reagent and buffer solution. The method therefore measured nitrate plus nitrite.

### 2.4.1 Reagent preparation

Hydrazine sulphate

0.075 g of hydrazine sulphate was added to 200 ml of deionized water in a 250 ml volumetric flask. 0.25 ml of 15% Brij-35 solution was also added. The neck of the flask was cleaned by adding more water and the solution was made up to the mark. The flask was stoppered to avoid reaction with atmospheric oxygen. Then the solution was mixed by a magnetic stirrer.

Catalyst solution

i copper sulphate stock solution

2.47 g of copper sulphate was dissolved in a 100 ml flask and made up to the mark. The solution was stored in a refrigerator.

ii working catalyst solution

1 ml of 2.47 % copper sulphate and 1 ml of 15 % Brij-35 were added to one litre deionized water and mixed.

Nitrate Nitrogen standard stock solution

7.218 g of dried potassium nitrate was dissolved in 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 solution.

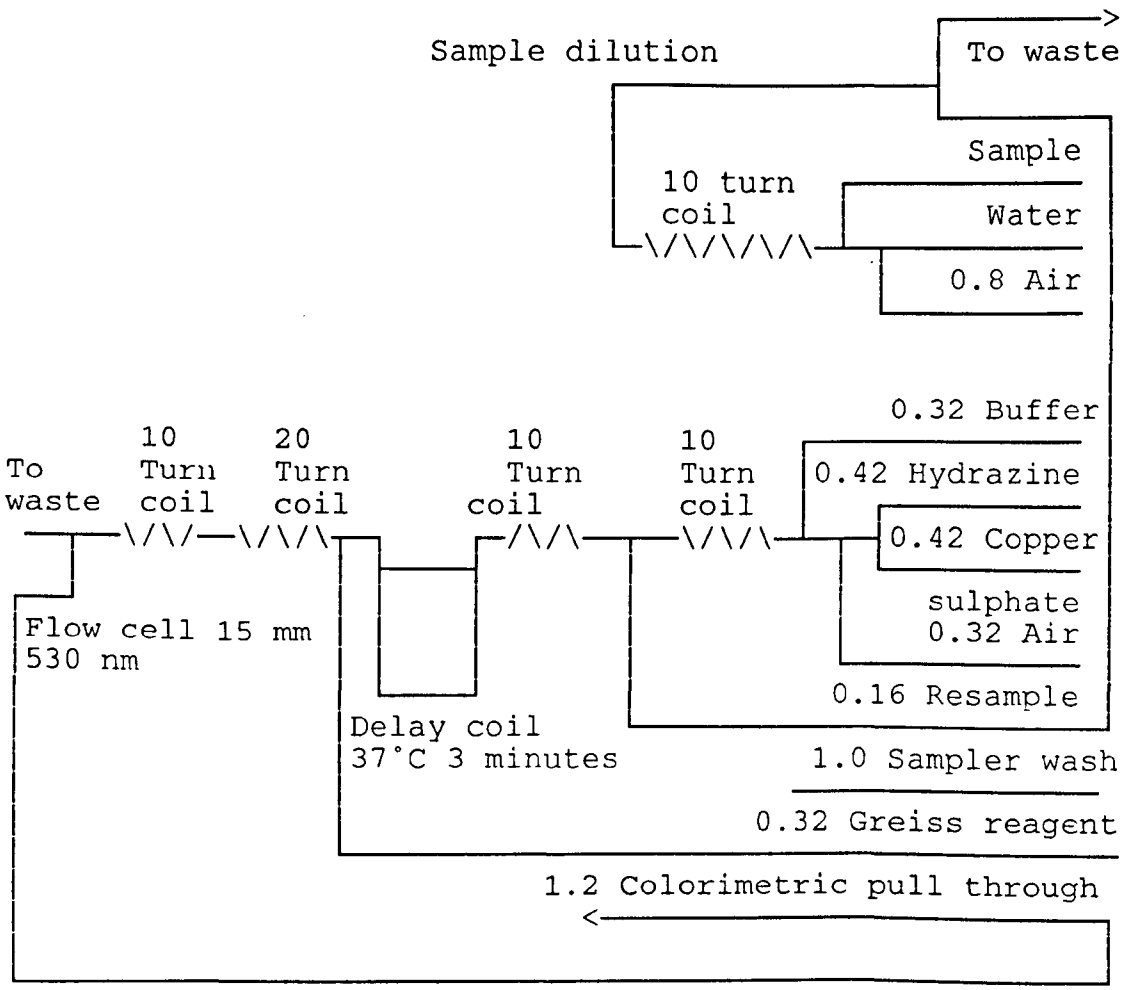



Figure 2.3 AutoAnalyzer Manifold For $NO_3$-N

2.4.2 Procedure

The manifold (Fig 2.3) was used for the determination of nitrate nitrogen in different extracting solutions. The samples were run at the rate of 40 per hour. The calibration for nitrate in the range of 0-5 mg $ml^{-1}$ was curved. Sample solutions having nitrate-N concentrations above were diluted in the range 0-5 mg $ml^{-1}$ using an in built dilution step.

## 2.5 DETERMINATION OF PHOSPHATE

The concentration of phosphorus in solution was determined spectrophotometrically by two methods: the Ascorbic acid/Molybdate method and the Molybdate/ Metavanadate method.

### 2.5.1 Ascorbic Acid/Molybdate Method

Phosphate was measured using the Technicon AutoAnalyzer II. The method is based on the formation of a phospho-molybdate complex which is reduced using ascorbic acid to give a blue colour which may be measured at 660 or 880 nm. In order to speed up the formation of the complex, a small amount of antimony is added. The intensity of the blue colour is proportional to the phosphorus concentration in the original solution. The method is applicable to water samples and a wide range of soil extract solutions and acid digests of plant or soil material. The schematic diagram of the flow system is shown in Figure 2.4.

#### 2.5.1.1 Reagents.

Acid ammonium molybdate.

60 ml concentrated sulphuric acid was added to 800 ml deionized water in the fume cupboard and cooled. 5.2 g ammonium molybdate was dissolved in the dilute acid solution. 0.1 g antimony potassium tartrate was dissolved in 100 ml of deionized water in a beaker. This solution was then added to the dilute acid ammonium molybdate solution by stirring with a glass rod to avoid precipitation. The

volume was made to 1 litre with degassed deionized water. The solution was stored in a dark glass bottle.

Ascorbic acid solution.

0.75 g ascorbic acid was dissolved in 100 ml of degassed deionized water.

Wetting agent solution.

0.5 ml Aerosol 22 was diluted to 1 litre with degassed deionized water.

Phosphate standard stock solution ( 1000 mg $l^{-1}$)

Potassium dihydrogen phosphate was dried in an oven at 110°C for one hour. 4.3937 g dried potassium dihydrogen phosphate was dissolved in deionized water in a beaker. The contents of the beaker were transferred to a 1 litre volumetric flask and diluted to the mark with deionized water.

Phosphorus working standard solutions 0-10 mg $ml^{-1}$

Phosphorus working standard solutions 0-10 mg $ml^{-1}$ were prepared containing 5 ml sulphuric acid plus 1 g or 2.5 g of sodium catalyst mixture or half Kjeltab ( containing potassium sulphate/copper sulphate/Se) per 100 ml.

Wash chamber solution: 50 ml $H_2SO_4$ $l^{-1}$

Neutralizing solution: 10 g of sodium hydroxide $l^{-1}$

2.5.1.2 Procedure

The filtrates were analysed for phosphate using the manifold shown in Figure 2.4 along with standard solutions, blanks and zeros. The samples were run at the rate of 40 per hour. The colour was developed in the water bath at 37 °C. The intensity of the colour was measured at 880 nm. The phosphate calibration graph is linear in the range of 0-5 mg $PO_4$-P $l^{-1}$. Samples having phosphate concentrations higher than 5 mg $l^{-1}$ were diluted by an inbuilt dilution system.

Manifold configuration for Kjeldahl digests

Diluter configuration

Sample: flow rate: 0.32 ml $min^{-1}$

Dilution 1.2 ml $min^{-1}$ 0.25 M NaOH (10 g $l^{-1}$)

Main manifold

Resample : flow rate 0.42 ml $min^{-1}$

Aerosol solution: flow rate 0.60 ml $min^{-1}$

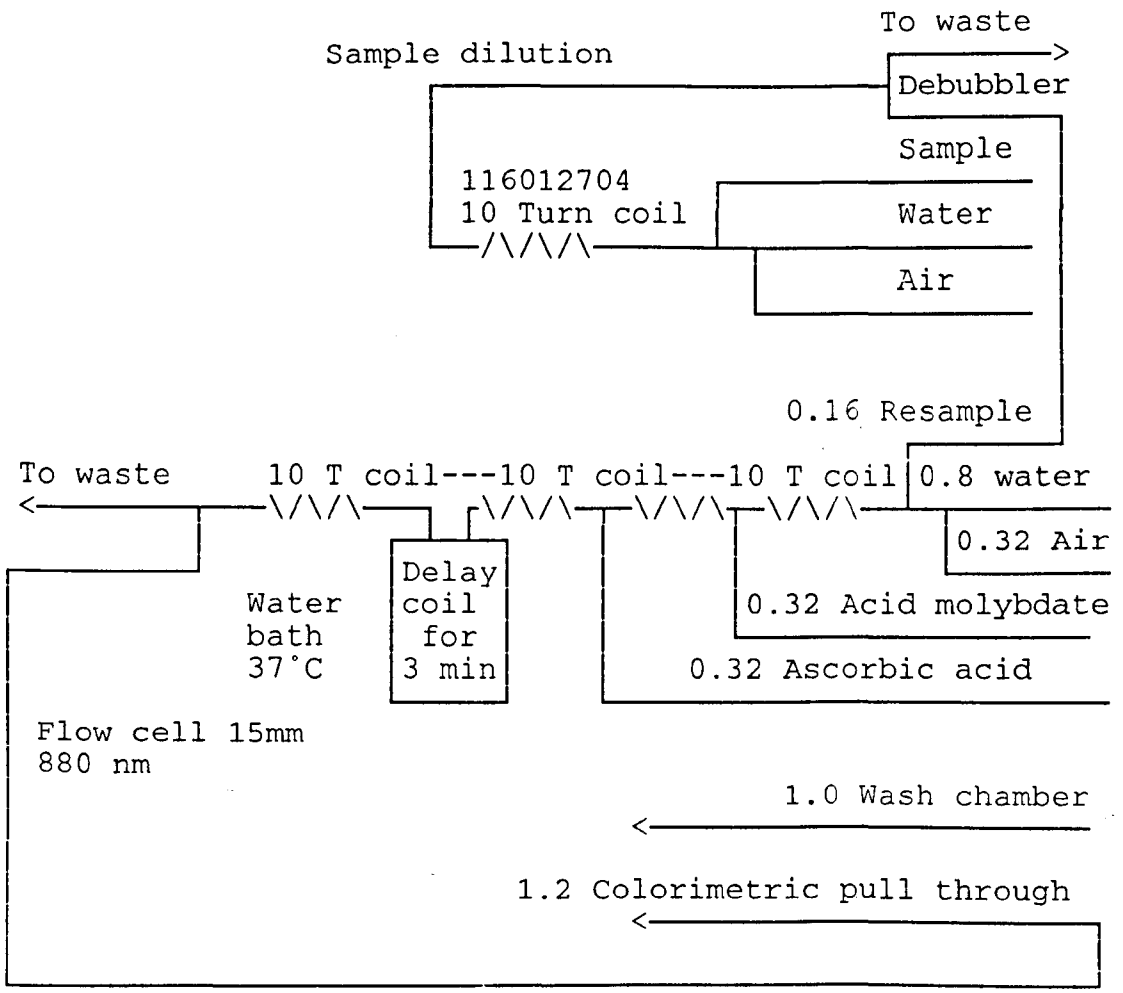



Figure 2.4 Technicon AutoAnalyzer II phosphate-P Ascorbic acid/ Molybdate method system

## 2.5.2. Molybdate/Metavanadate Method

Phosphate was determined on the Technicon Autoanalyser as the yellow phospho-vanado-molybdate complex at 420 nm in the range 0-10 mg $ml^{-1}$.

### 2.5.2.1 Reagents

Analytical grade reagents and deionized water were used throughout.

Ammonium molybdate- ammonium metavanadate

25 g of Ammonium molybdate and 1.25 g of Ammonium-metavanadate were dissolved in 900 ml of deionized water. 1 ml of Levor IV was added and the solution was made up to 1000 ml with deionized water and degassed.

Nitric acid

14 ml of Nitric acid were dissolved in 1000 ml of deionized water and degassed.

Phosphorus working standard solutions 0-10 mg $ml^{-1}$ were prepared containing 5 ml sulphuric acid plus 1 g, or 2.5 g of sodium salt mixture or half Kjeltab per 100 ml.

Wash chamber solution: 50 ml concentrated $H_2SO_4$ $l^{-1}$

Neutralizing solution: 10 g of sodium hydroxide $l^{-1}$

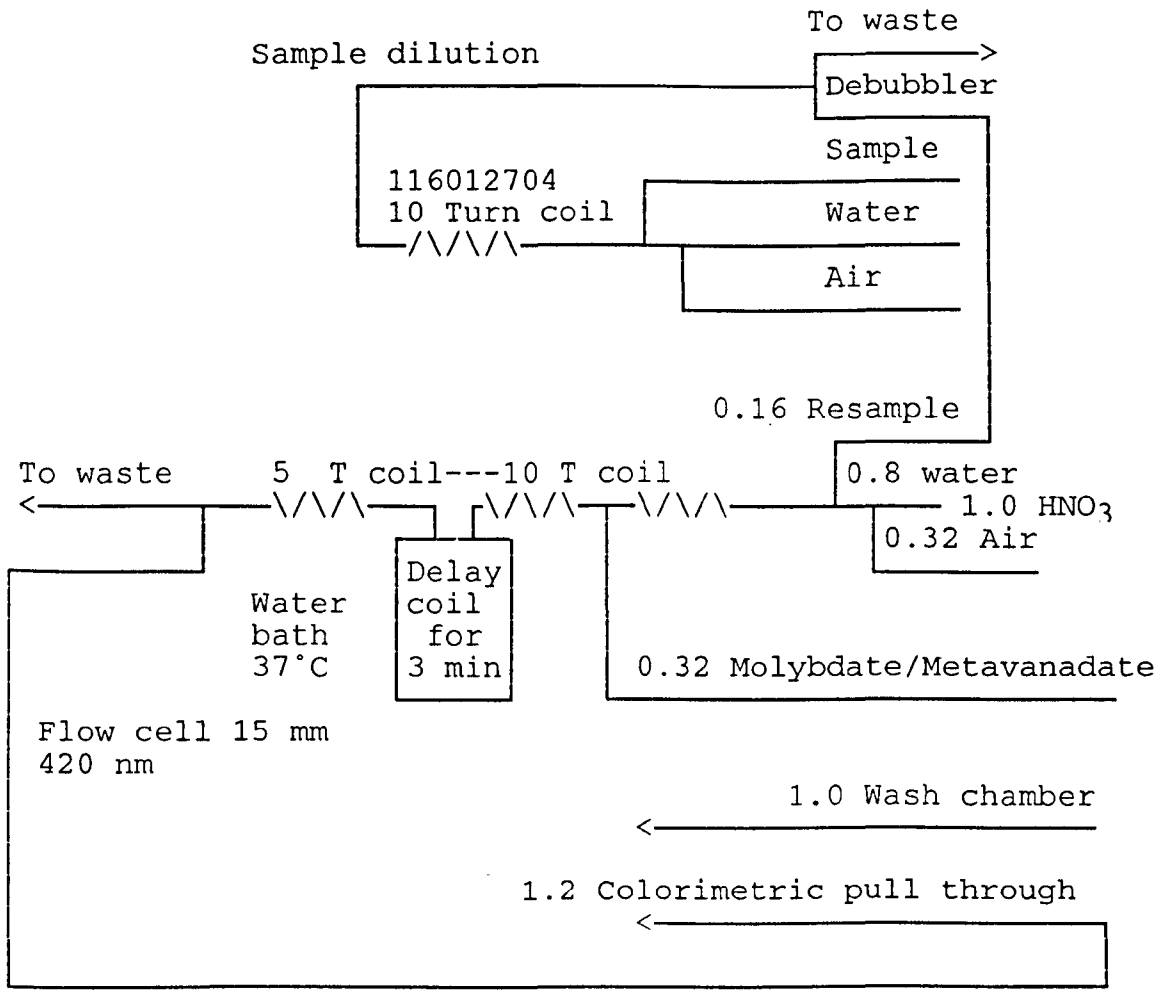



Figure 2.5 Technicon AutoAnalyzer II phosphate-P Molybdate/metavanadate method system

## 2.6 DETERMINATION OF POTASSIUM

The concentration of potassium in the diluted solution was determined with a flame photometer.

### 2.6.1 Reagents

Potassium stock standard solution 1 mg $ml^{-1}$.

Potassium-dihydrogen orthophosphate was dried at 102 °C for one hour and cooled in a desiccator. 1.740 g of the dried salt was dissolved in deionized water and diluted to 500 ml.

Potassium working standard solutions

0, 20, 40, 60 and 80 mg $l^{-1}$ standard solutions were prepared with the addition of 5 ml sulphuric acid plus 1 g or 2.5 g of sodium catalyst mixture per 100 ml deionized water.

## 2.7 EXTRACTION OF INORGANIC NITROGEN FROM SOIL

The inorganic nitrogen in soil (ammonium, nitrate and nitrite) can be determined once it has been extracted into solution. 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 in the sample, the extraction procedure needs to be modified because of the presence of ammonium absorbed on the colloidal complex of the soil.

Potassium sulphate salt solution was selected as an extractants in this study because the extract was satisfactory for use in the automated methods of analysis of nitrate and nitrite as well as ammonium used in this department. Potassium chloride solution interfered in the automated analysis of nitrate. Flowers and Arnold (1983) also used 0.5 M potassium sulphate during inorganic analysis of soil and they did not mention any problems.

Recently Khan (1987) recommended that shaking of soil in 0.5 M potassium sulphate at a soil solution ratio 2.5:50 (w:v) at a temperature of 2 °C for a period of 2 hours is the most suitable method of extraction for soil inorganic nitrogen analysis which is applicable to a broad range of soils.

### 2.7.1 Purification of 0.5 M potassium sulphate

174.25 g of 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 with 1 M 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 6.0 with 0.5 M $H_2SO_4$. Deionized water was added to allow for any loss of water during boiling due to evaporation. (Khan, 1987)

2.7.2 Washing of filter papers

0.5 M sulphuric acid was used for the washing of filter papers. Working in a fume cupboard 55 ml of concentrated $H_2SO_4$, measured by measuring cylinder, was added to 900 ml deionized water in a 1 litre volumetric flask. After cooling for half an hour the solution was made up to the mark.

The filter papers were folded separately into the plastic filter funnels. Two aliquots of 25 ml of sulphuric acid and five aliquots of 50 ml deionized water were used for the washing of filter papers (Shah, 1988).

After washing the filter papers in the funnels. The funnels containing paper were then allowed to dry for 4 hours in a 70 °C oven (Khan, 1987).

2.7.3 Procedure for extraction

2.5 g of soil was weighed into a 100 ml glass screw cap bottle and 50 ml of purified potassium sulphate solution was added with an automatic pipette. The glass bottle was capped with a plastic top and shaken for 2 hours at 2 °C on a box shaker.

## 2.8 STORAGE OF EXTRACTS

The filtered soil extracts were stored in a cold room at 2 °C prior to analysis. Khan (1987) recommended that if analysis is not possible immediately, extracts can be stored at 2 °C for two months.

## 2.9 DETERMINATION OF MOISTURE CONTENT OF SOIL

Porcelain basins were washed and cleaned and then left in the oven at 110 °C for 1 hour to dry. They were cooled in a desiccator and weighed. 10 g soil was weighed into each basin which was then placed for 24 hours in an oven at 110 °C, cooled in a desiccator and reweighed. The percent moisture content was determined on an oven dry basis:-

$$\% \text{ Moisture} = \frac{\text{weight fresh soil - weight oven dry soil}}{\text{weight oven dry soil}} \times 100$$

## 2.10 DETERMINATION OF MOISTURE CONTENT OF PLANT MATERIALS

2 g of plant material was weighed into a clean, dry and weighed basin. The basins were placed in the oven at 75 °C for 18 hours. The rest of the procedure for determining percentage moisture in plant material is similar to that for soil.

## 2.11 DETERMINATION OF SOIL ORGANIC MATTER

Following the determination of the moisture content sample basins were placed in the electric muffle furnace at 450 °C for 5 hours. The basins were transferred to an oven for 1 hour at 110 °C, then cooled in a desiccator and reweighed. The organic matter content was calculated as a percentage loss on ignition.

$$\% \text{ LOI} = \frac{\text{oven dry soil} - \text{ignited soil}}{\text{oven dry soil}} \times 100$$

## 2.12 DETERMINATION OF MOISTURE CONTENT AT -0.5 BAR.

Determination of the moisture content at -0.5 Bar soil moisture potential was carried out with the pressure plate apparatus. The three replicate samples of soil were placed on the plate and flooded with water. These were allowed to soak for 24 hours. The excess water was then removed from the plate which was then placed in the pressure plate apparatus and 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 percentage moisture content was determined on an oven dry basis.

## 2.13 MEASUREMENT OF SOIL pH

Soil pH was determined in a 5:1 water:soil mixture by a combined glass / reference electrode. The meter was first standardized with buffer solutions of pH 7.00 and 4.00. 2 g of air dried soil or an equivalent weight of fresh soil,

was taken in a glass vial and 10 ml of deionized water was added. The vial was shaken on a shaker for an hour. The soil suspension was stirred by swirling the electrode slightly and the pH read immediately.

## 2.14 MEASUREMENT OF SOIL CONDUCTIVITY

Soil conductivity was determined in a 5:1 water:soil mixture using a Jenway 4070 conductivity meter. It was determined in the same way as pH. 2 g of air dried soil, ground to pass a 4 mm sieve, was taken in a glass vial and 10 ml of water was added. The vial was shaken on a shaker for an hour. The soil suspension was filtered. The conductivity electrode was swirled in filtered solution and the conductivity value read when the reading stabilised.

## 2.15 SOIL MECHANICAL ANALYSIS

Particle size analysis for the determination of textural class was carried out by a modification of the MAFF method (1986),(Khan, 1987).

### 2.15.1 Reagents

i) Hydrogen peroxide 30%.

ii) Dispersing reagent (calgon)

25.0 g of sodium hexametaphosphate plus 3.5 g of sodium carbonate (anhydrous) were dissolved in water and diluted in to 500 ml.

iii) Silicon antifoaming agent

1 ml of 10 % aqueous emulsion was diluted into 100 ml water.

2 M HCL

170 ml of concentrated HCl was diluted with deionized water in a 1 litre volumetric flask and made up to the mark.

### 2.15.2 Procedure

The soils were passed through a 4 mm sieve and air dried. Approximately 10 g of each soil was weighed into separate 600 ml beakers. 10 ml of hydrogen peroxide and 2 drops of antifoaming agent were then added to each beaker. Once any initial reaction had ceased the beakers were heated on a steam bath for 30 minutes. The beakers were cooled and a further 10 ml of hydrogen peroxide added and

the beakers were heated and cooled once again. After cooling 10 ml of 2 M HCl was added to each beaker.

The soil suspension was then filtered through a Whatman No. 50 filter paper under vacuum, and rinsed with deionized water to remove soluble salts. The soil material was quantitatively recovered from the filter paper into the original beaker by washing with a jet of hot water and a spatula. The volume was adjusted to approximately 100 ml.

10 ml calgon solution was added to each beaker to aid in the dispersion of clays using an ultrasonic probe. The tip of the probe was set approximately 1 cm below the surface of the suspension which was stirred using a magnetic stirrer. Ultrasonic dispersion was then carried out for 5 minutes. After dispersion any soil adhering to the probe or magnetic stirrer bar was washed into the beaker using deionized water.

Fractionation of coarse and fine sand

The dispersed soil suspension were sieved through a 180 µm mesh sieve and a 53 µm mesh sieve into a 1 litre graduated cylinder. The 180 um mesh sieve was placed on top of the 53 um mesh sieve in a large funnel placed on the top of the cylinder. The suspension was carefully passed through the two sieves to trap the coarse plus medium sand on the top sieve and the fine sand on the lower sieve. The sieves were washed with deionized water to ensure complete separation of the two sand fractions. The contents of each sieve were transferred to weighed basins by washing with deionized water. The water was evaporated on a steam bath

and the basins were transferred to the oven for drying at 110 °C. The percentage of each sand fraction was calculated on oven dry soil basis.

Fractionation of silt and clay.

The cylinders were made up to the 1 litre mark with deionized water. Silt plus clay was fractionated by shaking each cylinder thoroughly for one minute to ensure that all the soil was in suspension. The cylinder was then placed on the bench and 50 ml of suspension was taken by pipette. The suspension was dried on a steam bath in weighed basins. The basins with dry material were dried overnight in an oven at 110 °C. The next day the basins were taken out, cooled then reweighed.

Fractionation of clay.

After removing the silt plus clay the cylinders were kept at a constant room temperature of 21 °C. The cylinders were shaken thoroughly then allowed to stand for 467 minutes. The appropriate settling time at 10 cm depth was 467 minutes. 25 ml of suspension was taken at a depth of 10 cm by placing each cylinder on a pipette stand. The suspension was dried and weighed and the clay content measured.

## 2.16 MEASUREMENT OF CATION EXCHANGE CAPACITY OF SOIL

Cation exchange capacity is a measure of the negatively charged sites on soil surfaces or the ability of the soil to hold plant available exchangeable cations. These cations are able to exchange with other cations in solution- the process of ion exchange. The cation exchange capacity provides information about the potential fertility of soil with regard to nutrient cations. It is also important to have information about the soil's ability to hold cations if the soil is considered as a repository for wastes - e. g. sewage sludge, mining wastes, industrial wastes or atmospheric fall-out.

### 2.16.1 Reagents

i) Potassium acetate solution.

5 litres of 1 M of potassium acetate was prepared by dissolving 490 g of potassium acetate in 5 litres deionized water.

ii) Ammonium acetate solution.

5 litres of 1 M of ammonium acetate was prepared by dissolving 385 g of ammonium acetate in 5 litres deionized water.

iii) Ethanol (95 %).

iv) Strontium chloride (5%).

50 g of strontium chloride ($SrCl_2$ $6H_2O$) were dissolved in a litre of deionised water.

Calcium working standard solutions 0 and 5 mg $ml^{-1}$.

Calcium working standard solutions 0 and 5 mg $l^{-1}$, were prepared by dilution of SpectrosoL 1000 mg $l^{-1}$ stock solution in 100 ml flasks containing 10 ml of 5 % strontium chloride and 10 ml of M ammonium acetate.

Magnesium working standard solutions 0 and 2 mg $ml^{-1}$.

Magnesium working standard solutions 0 and 2 mg $l^{-1}$, were prepared by dilution of SpectrosoL 1000 mg $l^{-1}$ stock solution in 100 ml flasks containing 10 ml of 5 % strontium chloride and 10 ml of M ammonium acetate.

Potassium and sodium working standard solutions.

The working standard solution for sodium 0, 1, 2, 3, 4, and 5 mg $l^{-1}$ and potassium 0, 2, 4, 6, 8 and 10 mg $l^{-1}$ were prepared in M ammonium acetate.

2.16.2 Procedure

Using a top balance, 10 g of air dried soil was weighed into a 100 ml beaker. The leaching column was plugged with glass wool and the soil was transferred into the leaching column. A small plug of glass wool was also used to plug the top of the column. A short piece of rubber tubing with a gate clamp was attached to the base of the column to control the flow rate. Duplicate columns were set up for each soil and used for the measurement of both CEC and exchangeable bases.

The columns were leached with 200 ml 1 M ammonium acetate at pH 7 and the leachate collected in a plastic bottle. The leachate was transferred to a 250 ml volumetric flask and made up to the mark with 1 M ammonium acetate solution. This was transferred to a plastic bottle and retained for the determination of exchangeable bases.

The columns were then leached with 200 ml of ethanol. This leachate was discarded. Finally the columns were leached with 1M potassium acetate at pH 7 and the leachate was collected in plastic bottle. The solution was transferred to a 250 ml volumetric flask and made up to the mark with 1M potassium acetate solution. This solution was retained for the measurement of CEC.

Measurement of Sodium and Potassium

Sodium and potassium were determined in the ammonium acetate leachate using the Flame photometer and standards prepared in M ammonium acetate.

Measurement of Calcium and Magnesium

Calcium and magnesium were measured by atomic absorption under standard conditions. Samples were diluted 10 times in volumetric flasks with the addition of 10 ml of 5 % strontium chloride solution.

Measurement of Ammonium

The concentration of ammonium in the molar potassium acetate leachates was measured using the Technicon Autoanalyser system.

The filtered solutions were analysed using the manifold shown in Figure 2.1 along with standard solutions, blanks and zeros. A 20 fold dilution step was incorporated into the manifold. The samples were run at the rate of 40 per hour and the colour was developed in the water bath at 37 °C.

Standard Range 0 - 50 $NH_4$-N mg $l^{-1}$

Diluter 0.1:2.0 Sample: dilution water.

# CHAPTER 3

# EVALUATION OF KJELDAHL DIGESTION METHOD

## 3.1 INTRODUCTION

The traditional method of measuring organically bound nitrogen is that of Dumas (1831) in which nitrogenous compounds were ignited with copper oxide in a stream of carbon dioxide and the gaseous products decomposed by copper into water, carbon dioxide and nitrogen gas. The latter is measured manometrically after absorption of carbon dioxide. Kjeldahl (1883) developed an alternative method involving the conversion of organic nitrogen into ammonium by boiling with sulphuric acid and distilling with alkali to liberate ammonia for determination by titration. The Kjeldahl process with certain modifications has been widely used for plant and soil analysis ever since. A few other methods of determining total nitrogen like the sulphuric acid and phosphoric acid mixture method of Honda (1962), the rapid chromic acid method of Flowers & Bremner (1991) and Sharma and Sud (1980) have been published but the most generally accepted is the Kjeldahl method.

The Kjeldahl procedures generally employed for the determination of total N involves two steps, ( Nelson & Sommers, 1973 ): i) digestion of the sample to convert organic and inorganic forms of N to ammonium-N and ii) determination of ammonium-N in the digest.

The total nitrogen recovery during digestion is influenced by the quantity of sample and sample size. Semimicro Kjeldahl digestion methods require a small quantity of sample, 50-200 mg plant material or 100-500 mg soil. Material must be finely ground before analysis. If plant material is ground to less than 40 mesh, a sample quantity of at least 100 mg is recommended to ensure that a representative subsample is analysed. Use of a 50 mg sample required that the plant material be ground to less than 80 mesh (Nelson and Sommers, 1980). To obtain reproducible results during semimicro Kjeldahl analyses of soils, it is normally necessary to grind the samples to less than 100 mesh. (Nelson and Sommers, 1980).

A second factor which influences the total nitrogen recovery is pretreatment of the sample to include nitrate nitrogen. Pretreatment of samples to include nitrate in total N determination is necessary because the routine Kjeldahl process fails to estimate nitrate and nitrite nitrogen. Three modifications of the Kjeldahl method have been used to recover nitrate and nitrite nitrogen in total Nitrogen analysis:- (1) The salicylic acid modification introduced by Cope (1916) where the sample is pre-heated with salicylic acid and sodium thiosulphate. (2) The alkaline reduction modification of Davisson and Parsons (1919) in which Devarda's alloy and alkali are used to reduce nitrate and nitrite to ammonia which is then collected in sulphuric acid and added to the digest. (3) The permaganate-reduced Iron modification of Olsen (1929) in which potassium permanganate is used to oxidise nitrite

to nitrate-Nitrogen which is then reduced to ammonium by reduced iron. The most popular method in use is the salicylic acid modification.

In the salicylic acid modification of the Kjeldahl method, the sample is treated with salicylic acid dissolved in concentrated sulphuric acid. The nitro compounds formed by the reaction of salicylic acid with nitrate in acid medium are reduced to the corresponding amino compounds by heating the mixture with sodium thiosulphate or Zn dust before digestion. The identity of the nitro compounds formed in this procedure have not been fully established, but work by Stalcup and William (1955) indicated that the main product of nitration is 5-nitrosalicylic acid and that small amounts of 3-nitrosalicylic acid are also formed. Although this method has been used extensively for total N analysis of soils containing nitrate and nitrite serious doubts have existed about its ability to recover nitrate quantitatively and its applicability to undried soils (Bremner, 1965). Goh (1972) increased doubt about the reliability of this procedure, because he reported that it gave poor recovery of nitrate nitrogen added to Muscatine soil. Results were significantly lower than those obtained by a reduced iron modification of the Kjeldahl method to include nitrate nitrogen. According to Piper (1947), nitration of salicylic acid cannot be applied successfully to undried soil samples. However, Bremner and Mulvaney (1982) suggested that good recovery of nitrate and nitrite can be obtained with the salicylic acid modification method in soils containing 0.6 ml of water per gram of soil. The

salicylic acid method of pre-digesting was slightly modified using sodium sulphate-catalyst mixture instead of potassium sulphate-catalyst mixture with selenium by Khan (1994) to facilitate the measurement of nitrogen, phosphorus and potassium in the same digest.

Bal (1925) found that the total nitrogen values obtained by Kjeldahl analysis of clay soils significantly increase with pretreating with water before digestion. Bremner and Harada (1959) showed that inclusion of a pretreatment with water had no effect in soils high in clay and clay fixed ammonium. Later Keeney and Bremner (1965) found that hydrogen fluoride pretreatment in Kjeldahl analysis of soils is an effective method for the inclusion of clay fixed ammonium.

A third factor which influences the total nitrogen recovery as reported by Nelson and Sommers (1980) is the acid mixture used for digestion. Concentrated sulphuric acid is normally the acid specified for digestion, although sulphuric/phosphoric acid mixtures have been used in semimicro-Kjeldahl procedures (Honda, 1962; Skjemstad & Reeve 1976 ). Batey et al. (1974) advocated use of an $H_2SO_4$-$HClO_4$ mixture for Kjeldahl analysis of plant materials, although the procedure has not been widely adopted. Other investigators have recommended use of hydrogen peroxide to assist in clearing the digests (Thomas et al., 1967; Parkinson & Allen, 1975) thereby decreasing digestion times. However, Nelson and Sommers (1973) found that sulphuric acid-hydrogen peroxide digestion procedures ( no potassium sulphate or catalysts were used ) produced

poor recoveries of total N from plant materials ( only 84% of the total N found by the standard procedure). In addition, Hambleton and Noel (1975) reported that hydrogen peroxide treatment resulted in excessive foaming and loss of sample from digestion tubes. There appears to be little advantage in the use of other acids or oxidants in conjunction with sulphuric acid for Kjeldahl digestion. However Hach et al. (1985) reported that results with a peroxymonosulphuric acid method are accurate, fast, and comparable to standard Kjeldahl methods.

A fourth factor is the addition of salt to raise the temperature. Various salts, such as potassium sulphate, sodium sulphate and phosphorus pentoxide have been added to Kjeldahl digestion mixtures to raise the boiling point of sulphuric acid. Digestion temperature increases from 332 °C with no salts to 371 °C when potassium sulphate is added at 1 g per ml sulphuric acid. High temperature digestion ensures recovery of nitrogen in compounds which do not decompose at the boiling point of concentrated sulphuric acid and markedly reduces the time required for complete digestion of samples. Some recently published semimicro-Kjeldahl methods do not specify use of salts in the digestion. However, Nelson and Sommers (1973) found that digestion mixtures without potassium sulphate gave significantly lower total N recoveries from plant materials than did mixtures with potassium sulphate.

The amount of salt specified per ml sulphuric acid in semimicro-kjeldahl procedures varies widely. High salt to acid ratios (more than 0.8 g potassium sulphate/ml $H_2SO_4$)

give high digestion temperatures and reduced times for complete digestion of the sample. However, sample handling difficulties increase with high salt ratios because mixtures tend to bump and splatter during digestion and digests are often difficult to dissolve prior to ammonium analysis. Furthermore, samples containing high salt concentrations may solidify during digestion, resulting in loss of nitrogen from the digests. Use of lower salt to acid ratios promotes easier digestion and analysis of digests, however, more time is required for complete sample digestion. Adding 0.33-0.5 g potassium sulphate per ml of sulphuric acid results in a digestion mixture which is easy to handle and which gives good recoveries of total N from soil and plant material ( Nelson and Sommers, 1980; Bremner and Mulvaney, 1982).

A fifth factor which influences the total N recovery is the use of catalysts. Wilfarth (1885) used copper sulphate and mercury oxide catalyst to hasten the reaction. Lauro (1931) introduced selenium as a catalyst and it has remained one of the most efficient means of hastening the reaction. Since that time copper, mercury and selenium have been the major catalysts used in the Kjeldahl digestion process to shorten the digestion time. Copper, generally in conjunction with limited amounts of selenium, is the most widely used catalyst for Kjeldahl analysis of soils. Mercury is more generally specified as the catalyst for total N determination of plant materials but it was reported by Sing (1984) that use of mercury catalyst causes significant interference due to precipitation of mercury in

the colorimetric determination by the Technicon Autoanalyzer. In addition to interference, mercury is also hazardous to health. Ashton (1936) compared the effect of selenium and copper sulphate on the time of digestion and found that selenium is effective in reducing digestion time. However, some early investigations suggest that selenium in the digest promotes volatile losses of nitrogen ( Davis & Wise, 1933). A number of investigations have not confirmed that selenium promotes N loss during digestion and have established the applicability of selenium as a catalyst in routine Kjeldahl determinations on biological materials ( Bremner, 1965; Nelson & Sommers, 1972 & 1973, Bremner and Mulvaney, 1982). In addition to interference and causing losses in nitrogen recovery, selenium is also hazardous to health.

A final factor which influences recovery is the digestion time. All the modifications of the Kjeldahl method recognize that digests must be at least "clear" if quantitative recovery of N in the sample is to be obtained. There are large differences in the boiling period after clearing which is recommended for various semimicro-Kjeldahl procedures. For digestion of plant materials, an after-boil period of 0-150 min has been specified. Nelson and Sommers (1973) found that a 1 hour after-boil period was required for maximum recovery of N from a variety of plant materials. Relatively long periods of digestion after clearing are required to recover fixed ammonium from soil samples. Bremner (1965) observed that a 5 hour after-boil period was required for some soils when a low $K_2SO_4$ : $H_2SO_4$

ratio was used. With the high $K_2SO_4$ : $H_2SO_4$ ratio a shorter period of boiling was required after clearing of digests. Nelson and Sommers (1972) found that a 3 hour after-boil period was required for maximum recovery of N in soils studied. Nelson and Sommers (1980) and Bremner and Mulvaney (1982) recommended at least 3 hour after clearing to ensure complete recovery of nitrogen.

The Kjeldahl procedure and most modifications for determining total nitrogen in soils and plants published prior to 1950 specified the use of macro-Kjeldahl flasks ( 350-800 ml ) for digestion and distillation. The introduction of aluminium blocks for Kjeldahl digestion in the early 1970s is a most noteworthy development. Nelson and Sommers (1972), Schuman et al., (1973), Gallaher et al., (1976) and Douglas et al., (1980) have described the use of block digester techniques for soil digestion.

Ammonium in digests of plants, soils and animal wastes can be measured by steam distillation, colorimetric methods and the ammonium electrode system. These methods have different degrees of sophistication, of sensitivity, cost of system and reagents, rapidity, accuracy and precision.

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. (Bremner, 1965; Bremner and Keeney, 1965 and Keen and Nelson, 1982.).

The determination of ammonium in the Kjeldahl digest of soil by the ammonia electrode has been reported by

Bremner and Tabatabai (1972). These electrodes on first consideration, appeared to 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 suitable meter electrode combinations easily exceeds the costs of distillation units or simple spectrophotometers.

The use of automated colorimetric analysis by the Technicon Autoanalyzer for the determination of inorganic nitrogen in various Kjeldahl digest solutions is attractive because large numbers of samples can be analysed quickly and with a high degree of reproducibility. In this method ammonium in the digest is separated from other digest constituents by continuous flow distillation or dialysis and determined by a procedure involving measurement of the colour of the indophenol complex formed by the Berthelot reaction. Alternateively it may be enough to simply dilute and neutralise the digests before colorimetric determination. Khan (1994) found that dilution and dialysis measured equal quantities of nitrogen in plant digests by the Technicon Autoanalyzer. Similarly Wang and Oien (1986) concluded that simple dilution of Kjeldahl digests for determining nitrogen at room temperature on the Autoanalyzer by the indophenol method is satisfactory.

The development of the Technicon Auto-Analyzer has provided a system capable of automating many analyses. Thomas et al., (1967) produced a paper concerned with the development of a single digestion procedure combined with

an automated system of analysis for N and P and spectrographic analysis for K in plant material. Phosphorus in the plant digest solutions may be determined spectrophotometrically by two methods. (1) Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid (phosphomolybdic acid) that is reduced to an intensely coloured molybdenum blue by ascorbic acid. (2) In a dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The concentration of P in solution is measured as the blue or yellow colour. Apostolatos (1984) published a rapid procedure for determination of phosphorus from a Kjeldahl digest. Blahemore et al., (1987) described the Autoanalyzer method as the most widely used in New Zealand for the determination of the main fractions of phosphorus. The automated method is labour conserving and can handle large numbers of samples on a routine basis with a high degree of reproducibility. It is also relatively more precise as each sample is treated in the same way. The human errors in mixing and other operations is reduced to a great extent. The automated procedure can also tolerate slight turbidity in the extract which is sometimes unavoidable. Lennox (1979) reported that the automatic determination of phosphate was an excellent procedure as regards recovery and effectiveness. The detection limit for the automated step was 1 ug P per litre.

The concentration of K in the diluted digest solution is determined with a flame photometer (Collins, 1952).

Keeping in view the above facts a study was undertaken to measure the nitrogen, phosphorus and potassium content in certified reference plant material and total nitrogen in soil samples of different textural class. The samples were digested by two methods, the standard Kjeldahl digestion method and the salicylic acid modification digestion method to compare the nitrate recovery. Each digestion method was applied with three salt/catalyst mixtures, 1 g and 2.5 g ( $Na_2SO_4$/ $CuSO_4$ ) and 2.5 g of Kjeltab to see the effect of salt/catalyst mixtures on digestion.

Nitrogen was measured by the steam distillation and the Technicon Auto-analyzer and phosphorus by the Ascorbic acid/Molybdate method and Molybdate/Metavanadate method to compare the precision and accuracy of each analytical method.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Materials

Two plant reference materials of Hay and Cabbage powder and two soil samples of Midelney and Dreghorn series were used. The description of each sample is as follows:-

Hay powder

Certified reference material No BCR 129 (hay powder) with individual identification No 0088 supplied by the Commission of the European Communities, Community Bureau of Reference was purchased from the Office of Reference Materials, Laboratory of the Government Chemist, Queens Road, Teddington, Middlesex TW11 0LY, UK.

Table 3.1 Certified values for total N P and K content of hay powder

| Element | Mass fraction based on dry mass | |
|---|---|---|
| | Certified value (mg/g) | Uncertainty(1) (mg/g) |
| Kjeldahl-N | 34.20 | 0.40 |
| Total N | 37.20 | 0.50 |
| Total P | 2.36 | 0.07 |
| Total K | 33.80 | 0.80 |

(1) The uncertainty is shown as the half width of the 95 % confidence interval of the mean.

Cabbage powder

Certified reference material No. GBW 08504 (cabbage powder) supplied by the Food Detection Science Institute Ministry of Commerce, Beijing, China was also obtained from the Laboratory of the Government of Chemist.

Table 3.2 Certified values for total N P and K content of Cabbage powder

| Element | Mass fraction based on dry mass | |
|---|---|---|
| | Certified value (mg/g) | SD(1) (mg/g) |
| Kjeldahl-N | 28.0 | 1.0 |
| Total P | 3.4 | 0.1 |
| Total K | 14.5 | 0.4 |

(1) Standard deviation

Dreghorn Series Soil

The soil sample site is situated at Barassie, Ayrshire, Scotland. The Grid reference 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 a freely drained brown forest soil.

Midelney Series Soil

The soil sample site is located at Bank Farm, Norfolk, England. The Grid reference is TF 588022. The soil was under permanent grass until 1979. Now it is used for arable crop cultivation. It belongs to the Midelney series

which is formed from a calcareous alluvial clay parent material. The series has been classed as a groundwater gley.

Table 3.3 Physical properties of soils (Khan,1987 & Mazumder,1992).

| | | Dreghorn Series | Midelney Series |
|---|---|---|---|
| LOI | (%) | 5.6 | 16.2 |
| pH water | (1:2.5) | 6.5 | 7.6 |
| Sand[1] | (%) | 87.9 | 17.8 |
| Silt | (%) | 10.9 | 40.3 |
| Clay | (%) | 1.2 | 42.3 |
| Texture class | | sand | clay |

1) Sand >53 µm. silt >2 µm clay <2 µm

3.2.2 Kjeldahl Digestion of Samples

Digestion of the samples was carried out by the standard Kjeldahl and the salicylic acid modification methods of Bremner and Mulvaney (1982).

3.2.2.1 Standard Kjeldahl Method

Reagents

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

Sulphuric acid.

Concentrated sulphuric acid with specific gravity 1.84.

Sodium sulphate-copper sulphate mixture

100 g sodium sulphate was finely ground with 10.0 g of copper sulphate in a mortar and pestle. This mixture was stored in a plastic bottle.

Kjeltabs

Kjeltabs (IB/61) from Thompson and Capper Limited, Runcorn, Cheshire, WA7 1NU England were used. Each tablet contains 5 g of the mixture (100 parts $K_2SO_4$ 6 parts $CuSO_4$ $5H_2O$ and 1 part Se).

Procedure

Before use the plant material was shaken for 2 minutes as per instructions provided by the supplier for rehomogenisation of the bottle contents. The air dried soil samples were ground in a mortar & pestle and passed through a 300 mesh sieve. They were then homogenised on a mechanical mixer for 1 hour.

Approximately 0.2 g of plant material, 0.5 g of Midelney series soil and 1 g of Dreghorn series soil was weighed for digestion. The samples were weighed on a boat made from aluminium foil. The boat was placed on the end of a pipette, pushed down to the end of a Tecator Kjeldahl tube and emptied. This was done to ensure that all the sample reached the base of the tube. The boat was reweighed

to allow correction for the small amount of sample remaining in the weighing boat.

Working in the fume cupboard a 5 ml aliquot of concentrated sulphuric acid and the catalyst salt mixtures ( see section 3.2.3 ) were added. The tubes were gently shaken to mix well. The 40 tube rack was placed in the Tecator block digester and heated at 375 °C for 3 hours. Following digestion, the tubes were removed from the block digester and left to cool until they were able to be handled. The digest was first made up to approximately 30 ml with deionized water and shaken well to dissolve all the digest. Then the digest was diluted to 100 ml in the graduated digestion tube.

After mixing, approximately 40 ml of the digest was stored in a plastic bottle for determination of the nitrogen content by steam distillation. The remaining portion of the digest was filtered through a Whatman No. 40 filter paper. The first 30 ml was discarded to wash the filter paper and the remaining 30 ml was collected and stored in a plastic capped glass vial for analysis on the Technicon Autoanalyzer.

### 3.2.2.2 Salicylic Acid Modification Method

#### Reagents

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

Salicylic acid-sulphuric acid mixture

25 g salicylic acid was dissolved in 1 litre of concentrated sulphuric acid in a beaker. The mixture was poured carefully into a 1 litre glass bottle fitted with a 5 ml acid dispenser.

Sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$)

500 g of sodium thiosulphate was obtained from FSA Laboratory Supplies, Bishop Meadow Road, Loughborough, LE11 0RG, England.

Sodium sulphate-copper sulphate mixture

100 g sodium sulphate was finely ground with 10.0 g of copper sulphate in a mortar and pestle. This mixture was stored in a plastic bottle.

Kjeltabs

Kjeltabs (IB/61) from Thompson and Capper Limited, Runcorn, Cheshire, WA7 1NU England were used. Each tablet contains 5 g of the mixture (100 parts $K_2SO_4$ 6 parts $CuSO_4$ $5H_2O$ and 1 part Se).

Procedure

The procedure for sample weighing and transferring to the Tecator tubes was the same as for the Standard Kjeldahl Digestion method.

A 5 ml aliquot of concentrated sulphuric acid-salicylic mixture was added to the sample and 0.5 g of sodium thiosulphate pentahydrate was also added. The tubes

were left to stand overnight. The tubes were then heated for 45 minutes at 135 °C, cooled, and the catalyst salt mixture ( see section 3.2.3 ) was added. The tubes were gently shaken to mix well. The 40 tube rack was placed in the Tecator block digester and heated at 375 °C for 3 hours. Following digestion the tubes were removed from the block digester and left to cool until they were able to be handled. The digest was first made up to approximately 30 ml with deionized water and shaken well to dissolve all digest. Then the digest was diluted to 100 ml in the graduated digestion tube. The procedure for filtration and storage was also the same for both methods.

### 3.2.3 Experimental Design

Experiment consisted of the analysis of 4 experimental materials using 2 digestion methods and 3 catalyst mixtures.

Experimental materials

1 Certified hay powder.

2 Certified cabbage powder.

3 Midelney series soil.

4 Dreghorn series soil.

Digestion Methods

1 Standard Kjeldahl Method.

2 Salicylic Acid Modification Method.

Catalyst treatments

1. $Na_2SO_4/CuSO_4$:- 1 g of sodium sulphate and copper sulphate pentahydrate mixture (10:1 by weight).

2. $Na_2SO_4/CuSO_4$:- 2.5 g of sodium sulphate and copper sulphate pentahydrate mixture (10:1 by weight).

3. Kjeltabs :- 2.5 g of potassium sulphate, copper sulphate pentahydrate and selenium (100:6:1 by weight).

Replications 5 replicates of each treatments were digested.

Blanks 3 blanks were used where no sample material was added to the digestion procedure. This was repeated for each of the catalyst treatments.

Fifteen tubes were allotted to each of the two sample materials and nine to blanks. Each tube was randomly placed into the holes of the block digester. The random number was allotted to each tube by a computer program.

Ammonium-Nitrogen in the digests was measured by steam distillation and Technicon Autoanalyzer. Phosphorus was measured by the Technicon Autoanalyzer and potassium by flame photometer.

## 3.2.4 Determination of Major Plant Nutrients in the Digests

### 3.2.4.1 Ammonium

Determination of the concentration of ammonium nitrogen in the digest was carried out by steam distillation as described in Chapter two section 2.2.1 and by the Technicon Autoanalyzer system as described in section 2.2.2 with certain modifications as follows:-

Preparation of working standard solution and reagents

In addition to the standard stock solution ( 1000 N mg $l^{-1}$ ) and reagents for the ammonium manifold as per Chapter 2 section 2.2.2.1, the following working standards and solutions were prepared for ammonium determination in plant and soil digests.

Preparation of working standards

Working standards were prepared at 0 and 100 mg N $l^{-1}$ for the analyses of plant material digests and at 0 and 50 mg N $l^{-1}$ for the analysis of soil digests. The standards contained the same concentration of acid and catalyst mixture as the digests. The appropriate weight of catalyst mixture was added to 5 ml sulphuric acid and heated at 375 °C on the Tecator block digester until dissolved. The solution was cooled and diluted with 60 ml of deionized water. The diluted solutions were transferred into the volumetric flasks. The solutions containing Kjeltabs were filtered through a Whatman filter paper No. 40 before transfer to the volumetric flask. An appropriate volume of

1000 N mg $l^{-1}$ $NH_4^+$ stock solution was added and the volume made up to 100 ml.

Additional Reagents

a) Wash chamber solution: 50 ml of concentrated sulphuric acid was added to 800 ml of water in 1 litre bottle and made up to the mark.

b) Dilution /neutralizing solution: 3.6 g of sodium hydroxide was dissolved in a litre of deionized water.

Procedure

The filtered solutions were analysed directly on a Technician Autoanalyzer II system. Forty samples per hour were run with a dilution /neutralisation step before the main manifold.

i Sample wash solution 5 % v/v $H_2SO_4$

ii Dilution ratio 20:1

| | |
|---|---|
| sample | 0.1 ml/minute |
| dilutent | 2.0 ml/minute |
| air | 0.8 ml/minute |

3.2.4.2 Phosphorus

Determination of phosphorus concentration in the digests was carried out by two methods, the Ascorbic acid/Molybdate method and Molybdate/Metavanadate method as in section 2.5.1 and section 2.5.2.

3.2.4.3 Potassium

Determination of potassium concentration was done by flame photometer as in section 2.6.

## 3.2.5 Determination of Nitrate

3.2.5.1 Method of nitrate extraction in plant materials

0.5 g of hay and cabbage were weighed in five replicates and shaken for one hour with 50 ml of deionized water containing 1 ml per litre of Brij-35 wetting agent. The extract was filtered through a Whatman No 1 filter paper. The extracts were diluted in a ratio of 1:50 prior to analysis.

3.2.5.2 Determination of nitrate in extracts of plant material

The concentration of nitrate in the extracts of hay and cabbage was measured by a Technicon Autoanalyzer system as per section 2.4.

3.2.5.3 Method of nitrate extraction in soil

The nitrate was extracted from soils by the method described in Chapter 2 section 2.7

3.2.5.4 Determination of nitrate in soil extract

The concentration of nitrate in the soil extracts was measured by Technicon Autoanalyzer system by the method described in Chapter 2 section 2.4.

### 3.2.6 Statistical Analysis.

The Minitab programme version 7.2 was used for statistical analysis of the data.

The General Linear Model and Fisher LSD test was used for analysis of variance.

A t test was used to compare results with the certified values and a paired t test was used to compare individual digests analysed by two different analytical meathods.

## 3.3 RESULTS AND DISCUSSION

This study was carried out to evaluate the Kjeldahl digestion method for determining total nitrogen, phosphorus and potassium in plant materials and total nitrogen in soils. Digestion was carried out for 3 hours after clearing at a constant temperature of 375 °C on a Tecator aluminium block digester. The results obtained are shown in Tables 3.4 to 3.19. All the results are reported on an oven-dry basis.

### 3.3.1 Nitrogen

#### 3.3.1.1 Effect of catalyst mixture and digestion methods on total nitrogen recovery from certified plant powders.

The results are presented for the determination of total nitrogen in the digests by Technicon Auto-Analyzer and steam distillation in Tables 3.4 to 3.7. Discussion of the effects of catalyst mixture and digestion methods are confined to the results from the analysis by Technicon Auto-Analyzer II (Comparison of the results obtained by the Technicon Auto-Analyzer and steam distillation are discussed at the end of this section 3.3.1).

Table 3.4 Effect of catalyst mixture and digestion method on total nitrogen determination in certified Hay powder (CRM 129) measured by Technicon Auto- Analyzer.

| Catalyst Mixture | | Total Nitrogen Content (mg/g) | | | |
|---|---|---|---|---|---|
| Salt/Catalyst | weight (g) | Standard Kjeldahl | | Salicylic Modification | |
| $Na_2SO_4/CuSO_4$ | 1.0 | 33.5 a[1] | *[2] | 35.3 bc | ** |
| $Na_2SO_4/CuSO_4$ | 2.5 | 35.1 bc | ** | 35.3 bc | * |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 34.9 b | * | 35.7 c | * |

1 Figures with the same letter following are not significantly different at the 5 % level using a Fisher LSD Test.
2 Values significantly different from the certified value ( 34.2 mg/g ) using a t test are indicated as follows * 5% level, ** 1% level.

The size of a significant difference from the certified value using the t test depends on the variability of individual digestion techniques, thus in some cases a small difference may be more significant than a large difference using an alternative method.

Table 3.5 Effect of catalyst mixture and digestion method on total nitrogen determination in certified Hay powder (CRM 129) measured by steam distillation titration.

| Catalyst Mixture | | Total Nitrogen Content (mg/g) | | | |
|---|---|---|---|---|---|
| Salt/Catalyst | weight (g) | Standard Kjeldahl | | Salicylic Modification | |
| $Na_2SO_4/CuSO_4$ | 1.0 | 34.4 a[1] | NS[2] | 35.4 bc | * |
| $Na_2SO_4/CuSO_4$ | 2.5 | 35.2 bc | NS | 35.7 c | ** |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 34.6 ab | NS | 35.2 bc | NS |

1 Figures with the same letter following are not significantly different at the 5 % level using a Fisher LSD Test.

2 Values significantly different from the certified value ( 34.2 mg/g ) using a t test are indicated as follows * 5% level, ** 1% level and NS indicate value is nonsignificant from certified value.

Table 3.6 Effect of catalyst mixture and digestion methods on total nitrogen determination in certified powder of Cabbage (GBW 08504) measured by Technicon Auto-Analyzer.

| Catalyst Mixture | | Total Nitrogen Content (mg/g) | | | |
|---|---|---|---|---|---|
| Salt/Catalyst weight | | Standard | | Salicylic | |
| | (g) | Kjeldahl | | Modification | |
| $Na_2SO_4/CuSO_4$ | 1.0 | 28.1 a[1] | NS[2] | 30.4 d | *** |
| $Na_2SO_4/CuSO_4$ | 2.5 | 29.3 b | ** | 29.7 c | * |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 29.2 b | ** | 29.3 b | ** |

1. Figures with the same letter following are not significantly different at 5 % level using a Fisher LSD Test.
2. Values significantly different from the certified value ( 28.0 mg/g ) using a t test are indicated as follows * 5% level, ** 1% level, *** 0.01% level and NS indicate value is nonsignificant from certified value.

Table 3.7 Effect of catalyst mixture and digestion methods on total nitrogen determination in certified powder of Cabbage (GBW 08504) measured by steam distillation titration.

| Catalyst Mixture | | Total Nitrogen Content (mg/g) | | | |
|---|---|---|---|---|---|
| Salt/Catalyst weight | (g) | Standard Kjeldahl | | Salicylic Modification | |
| $Na_2SO_4/CuSO_4$ | 1.0 | 28.4 a[1] | NS[2] | 30.9 c | *** |
| $Na_2SO_4/CuSO_4$ | 2.5 | 28.3 a | * | 29.9 b | * |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 29.2 b | ** | 29.7 b | ** |

1. Figures with the same letter following are not significantly different at 5 % level using a Fisher LSD Test.

2. Values significantly different from the certified value ( 28.0 mg/g ) using a t test are indicated as follows * 5% level, ** 1% level, *** 0.01% level and NS indicate value is nonsignificant from certified value.

Tables 3.4 and 3.6 show the effect of catalyst mixture and digestion methods on the determination of total nitrogen in hay and cabbage powder. For both hay and cabbage in the standard Kjeldahl method significantly lower amounts of total nitrogen were recovered by the treatment containing 1 g of catalyst mixture compared to 2.5 g catalyst mixture and Kjeltabs. The difference between the amount of total nitrogen measured in the standard Kjeldahl digestion method and the salicylic acid modification method with 2.5 g of catalyst mixture was non significant. The effect of catalyst mixture in the Salicylic acid

modification was non significant for hay whereas a small effect of catalyst mixture was measured for cabbage.

To test the reliability of the methods, the results were compared with the certified values of Kjeldahl nitrogen. As Tables 3.4 and 3.6 show, recovered values of Kjeldahl nitrogen in hay and cabbage were significantly different from the certified values. With the exception of the 1 g of catalyst mixture used with the standard digestion method, the measured values for Kjeldahl nitrogen were significantly greater than the respective certified values. For hay powder Kjeldahl nitrogen recovered with 1 g catalyst mixture with the standard method was significantly less than the certified value whereas in cabbage the recovered nitrogen was not significantly different to the certified value.

The reason for the low recovery with 1 g catalyst in the standard Kjeldahl method may be incomplete digestion and the higher recovery of Kjeldahl nitrogen with respect of certified value in standard method with 2.5 g catalyst may be the partial recovery of nitrate. To confirm the nitrate content in the samples nitrate was extracted from samples and the results are shown in Table 3.8.

Table 3.8 Extractable Nitrate Nitrogen in Plant materials

| Material | Nitrate-N (mg/g) |
|---|---|
| Hay Powder | 3.1 |
| Cabbage Powder | 3.2 |

The nitrate content in hay and cabbage powder was 3.1 and 3.2 mg/g, respectively, which is approximately 10 % of total Kjeldahl nitrogen (the same amount of nitrate has been reported by the material certifying agency). It is clear that a significant amount of nitrate is present in the powders. The difference between the results of nitrogen recovery with the standard Kjeldahl method and the salicylic acid modification were not of this magnitude. Ignoring the 1 g of catalyst mixture treatment the difference between both methods of digestion was not significant for hay and a small effect was observed for cabbage powder. So it is clear that the standard method is recovering part of the nitrate and the salicylic acid modification method is not recovering all of the nitrate. These partial recoveries of the nitrate are increasing the measured values of Kjeldahl-N compared to the certified values.

Nelson & Sommers (1973) evaluated a standard digestion procedure ( 1.1 g of catalyst mixture of 100 g potassium sulphate, 10 copper sulphate and 1 g selenium in

4 ml of sulphuric acid ) and a Salicylic acid digestion procedure ( 1.1 g of above catalyst mixture in 4 ml of salicylic-sulphuric acid mixture of 5 g : 200 ml ) on the recovery of nitrate nitrogen from plant material. They showed similar total N values for both methods for samples containing low levels of nitrate. However, the Salicylic modification yielded higher total N values for samples containing 0.35 and 0.31% nitrate nitrogen. They further evaluated nitrate nitrogen recovery by both methods with added potassium nitrate and reported that recovery of added nitrate by the Salicylic acid method ranged from 95 to 100 % whereas for the standard method it was between 16 and 55% of the added nitrate-N. They also concluded that the standard method recovered 21-55% of the nitrate naturally present in the plant material.

The other reason for the higher recoveries in the present study is the digestion method used for certification. The method used by the Commission of European Communities, Community Bureau of Reference for determining nitrogen in the certified plant material was described as follows. The certified plant material was digested in a 50 ml Kjeldahl flask with 5 ml sulphuric acid, 3 ml $H_2O_2$ and 50 mg selenium as a catalyst, boiling over glass beads for 1.5 to 3.5 h until clear; after completion of dissolution, dilution of the cooled digest to 50 ml with distilled water; mixing and filtering through filter paper; steam distillation of 10 ml of digest for 5 minutes in a Markham unit after addition of 15 ml NaOH;

absorption of $NH_3$ in a 1% $H_3BO_3$ solution; verification of the alkalinity at the end of the distillation and finally titrating with acid. Nelson & Sommers (1973) reported that various methods using $H_2O_2$-$H_2SO_4$ digestion gave poor recoveries of N in plant materials ( 84 % N was recovered by $H_2O_2$-$H_2SO_4$ with 1.1 g of catalyst mixture in 4 ml of sulphuric acid). Similarly Singh et al. (1984) measured higher nitrogen values by catalyst digestion than with hydrogen peroxide accelerated Kjeldahl digestion.

3.3.1.2 Effect of catalyst mixture and digestion method on total nitrogen recovery from soils

Tables 3.9 to 3.12 show the effect of catalyst mixture and digestion method on the determination of total nitrogen in Midelney and Dreghorn series soils.

The soil samples also show significantly lower total nitrogen recovery by the standard Kjeldahl digestion method with the treatment containing 1 g of catalyst mixture compared to 2.5 g catalyst mixture and Kjeltabs. The difference between 2.5 g and Kjeltab in standard method is non significant in both soils. The salicylic acid modification method shows lower recovery of nitrogen than the standard Kjeldahl method in the Dreghorn soil samples. In the Midelney soil samples nitrogen recovery was not significantly different in the 1 g catalyst mixture using the standard Kjeldahl digestion and all three treatments of the salicylic acid modification method.

Table 3.9 Effect of catalyst mixture and digestion methods on total nitrogen determination on Midelney series soil measured by Technicon Auto-Analyzer

| Catalyst Mixture | | Total Nitrogen Content (mg/g) | | | |
|---|---|---|---|---|---|
| Salt/Catalyst weight | (g) | Standard Kjeldahl | | Salicylic Modification | |
| $Na_2SO_4/CuSO_4$ | 1.0 | 7.19 | ab[1] | 7.23 | bc |
| $Na_2SO_4/CuSO_4$ | 2.5 | 7.49 | d | 7.21 | bc |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 7.38 | cd | 7.01 | a |

1 Figures with the same letter following are not significantly different at the 5 % level using a Fisher LSD Test.

Table 3.10 Effect of catalyst mixture and digestion methods on total nitrogen determination on Midelney series grass soil measured by steam distillation titration.

| Catalyst Mixture | | Total Nitrogen Content (mg/g) | | | |
|---|---|---|---|---|---|
| Salt/Catalyst | weight (g) | Standard Kjeldahl | | Salicylic Modification | |
| $Na_2SO_4/CuSO_4$ | 1.0 | 7.07 | abc[1] | 7.05 | abc |
| $Na_2SO_4/CuSO_4$ | 2.5 | 7.24 | c | 6.95 | ab |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 7.15 | bc | 6.85 | a |

1 Figures with the same letter following are not significantly different at the 5 % level using a Fisher LSD Test.

Table 3.11 Effect of catalyst mixture and digestion methods on total nitrogen determination on Dreghorn series soil measured by Technicon Auto-Analyzer

| Catalyst Mixture | | Total Nitrogen Content (mg/g) | | | |
|---|---|---|---|---|---|
| Salt/Catalyst | weight (g) | Standard Kjeldahl | | Salicylic Modification | |
| $Na_2SO_4/CuSO_4$ | 1.0 | 2.15 | c[1] | 2.11 | bc |
| $Na_2SO_4/CuSO_4$ | 2.5 | 2.24 | d | 2.07 | ab |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 2.28 | d | 2.01 | a |

1 Figures with the same letter following are not significantly different at the 5 % level using a Fisher LSD Test.

Table 3.12 Effect of catalyst mixture and digestion methods on total nitrogen determination on Dreghorn series soil measured by steam distillation titration

| Catalyst Mixture | | Total Nitrogen Content (mg/g) | |
|---|---|---|---|
| Salt/Catalyst weight | (g) | Standard Kjeldahl | Salicylic Modification |
| $Na_2SO_4/CuSO_4$ | 1.0 | 1.99 a[1] | 1.97 a |
| $Na_2SO_4/CuSO_4$ | 2.5 | 2.13 b | 1.98 a |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 2.15 b | 1.97 a |

1 Figures with the same letter following are not significantly different at the 5 % level using a Fisher LSD Test.

The effect of the three catalyst mixtures in the salicylic acid modification shows a downward trend of nitrogen recovery from 1 g catalyst mixture to 2.5 g and Kjeltabs. The standard Kjeldahl method containing 2.5 g of catalyst mixture is recovering significantly higher total nitrogen compared to the salicylic acid modification in both soil series. The lowest nitrogen was recovered in a treatment containing Kjeltabs in the salicylic acid modification in both soils.

The nitrate content of the two soil samples was not a significant proportion of Kjeldahl nitrogen as shown in Table 3.13. The nitrate content in the Dreghorn soil was higher than in the Midelney soil series. It is difficult to

see whether the salicylic acid modification method is recovering nitrate nitrogen or not as the values for total nitrogen recovered are lower than with the standard Kjeldahl method. The standard deviation of the total nitrogen is higher than the nitrate content in both soil series.

Table 3.13 Extractable inorganic nitrogen in soil samples

| Soils series | Ammonium-N (mg/g) | Nitrate-N (mg/g) | Nitrite-N (mg/g) |
|---|---|---|---|
| Midelney | 0.022 | 0.037 | 0.001 |
| Dreghorn | 0.016 | 0.057 | 0.002 |

The reason for the low nitrogen recovery with the 1 g catalyst mixture from soil samples is incomplete digestion, as for the plant material. The reason for the downward trend of nitrogen recovery (from 1 g to 2.5 g catalyst mixture and to Kjeltab) in the salicylic acid method is the high salt content ( 2.5 sodium sulphate or potassium sulphate with 0.5 g of sodium thiosulphate / 5 ml of sulphuric acid). Similarly Bremner (1960) reported that the main disadvantage of using high salt content is the risk of loss of nitrogen during digestion. The presence of selenium in the Kjeltabs with sodium thiosulphate in the salicylic acid modification caused N losses with high salt content. In the past several authors have reported N loss when using selenium as a catalyst. ( Ashton, 1936; and Bremner, 1960).

### 3.3.1.3 Comparison of steam distillation-titration with Technicon Auto-Analyzer method

The manual steam distillation-titration method of determining ammonium nitrogen involves liberating ammonia with the flow of steam from the alkaline solution into the boric acid indicator mixture solution. The quantity of ammonium was estimated by titrating the indicator mixture with acid. The details of the procedure and reagents is in Chapter 2, section 2.2.1.

The Technicon Auto-Analyzer method of determining ammonium nitrogen is based on the change in colour developed by reacting with phenol under alkaline conditions and measured in a colorimeter. The complexing reagent is used to prevent interferences and to increase sensitivity. The details of the method is in Chapter 2, section 2.2.2

The methods were compared by using the Kjeldahl digests of certified hay and cabbage powders and Midelney and Dreghorn series soils. Table 3.14 shows the means and standard deviations of the measured values of total nitrogen by the steam distillation method and the Technicon Auto-Analyzer in hay, and cabbage powders, and Midelney and Dreghorn series soils.

Table 3.14 Comparison of the means and standard deviations of measured nitrogen by steam distillation and Technicon Auto-Analyzer.

| Sample | Steam distillation | | Technicon Auto-Analyzer | |
|---|---|---|---|---|
| | Means | Sd | Means | Sd |
| | ..(mg N /kg)... | | ....(mg N /kg).. | |
| Hay | 34.9 | 0.9 | 35.0 | 0.8 |
| Cabbage | 29.4 | 1.1 | 29.2 | 0.9 |
| Midelney | 7.23 | 0.17 | 7.26 | 0.17 |
| Dreghorn | 2.08 | 0.10 | 2.09 | 0.10 |

The means of the measured values by both methods show similar amounts of nitrogen in hay and cabbage powders, and in Midelney and Dreghorn series soils. The standard deviations of the steam distillation method and the Technicon Auto-Analyzer method are the same. Statistically there is no significant difference between the two analytical techniques by applying a paired t test on the data for hay and cabbage powders, and the Midelney and Dreghorn soils. Both methods achieve comparable ammonium values and, although the time saved is confined to the second step of the final N determination it is substantial. The rate of estimating ammonium by steam distillation method was 12 samples per hour while the Technicon Auto-Analyzer analysed 40 samples per hour. The Technicon Auto-Analyzer method is labour conserving and can handle a large number of samples on a routine basis with a high degree of reproducibility. It is also relatively more precise as each sample is treated in the same way.

### 3.3.2 Phosphorus

3.3.2 Effect of catalyst mixture and digestion methods on phosphorus recovery from certified plant powders.

Tables 3.16 to 3.19 show the effect of catalyst mixture and digestion method on the determination of phosphorus in hay and cabbage powder. The effect of catalyst mixture and digestion method on the phosphorus determination was non significant for both methods of determination, the Molybdate/Metavanadate Method and the Ascorbic acid/Molybdate Method. The only exception was in the results for hay powder measured by the Ascorbic acid/Molybdate method. Treatment with 1 g and 2.5 g of catalyst mixture in the salicylic acid modification recovered a lower amount of phosphorus than other treatments. Similarly Alfonso et al. ( 1991 ) found that the amount of sodium sulphate and copper sulphate with Se and time of digestion did not affect the determination of total P in Kjeldahl digests of plant material.

Table 3.16 Effect of catalyst mixture and digestion methods on total phosphorus determination in certified powder of Hay (CRM 129) by Ascorbic acid/Molybdate Method.

| Catalyst Mixture | | Total Phosphorus Content (mg/g) | | | |
|---|---|---|---|---|---|
| Salt/Catalyst | weight (g) | Standard Kjeldahl | | Salicylic Modification | |
| $Na_2SO_4/CuSO_4$ | 1.0 | 2.61 bc[1] | ***[2] | 2.54 a | *** |
| $Na_2SO_4/CuSO_4$ | 2.5 | 2.65 c | ** | 2.55 ab | *** |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 2.66 c | *** | 2.64 c | *** |

1 Figures with the same letter following are not significantly different at 5 % level using a Fisher LSD Test.

2 Values significantly different from the certified value ( 2.36 mg/g ) using a t test are indicated as follows ** 1% level, *** 0.01% level.

Table 3.17 Effect of catalyst mixture and digestion methods on total phosphorus determination in certified powder of Hay (CRM 129) by Molybdate/Metavanadate Method.

| Catalyst Mixture | | Total Phosphorus Content (mg/g) | |
|---|---|---|---|
| Salt/Catalyst weight | (g) | Standard Kjeldahl | Salicylic Modification |
| $Na_2SO_4/CuSO_4$ | 1.0 | 2.69 a[1] ***[2] | 2.71 a *** |
| $Na_2SO_4/CuSO_4$ | 2.5 | 2.76 a ** | 2.76 a *** |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 2.70 a *** | 2.73 a *** |

1 Figures with the same letter following are not significantly different at 5 % level using a Fisher's LSD Test.

2 Values significantly different from the certified value ( 2.36 mg/g ) using a t test are indicated as follows ** 1% level, *** 0.01% level.

Table 3.18 Effect of catalyst mixture and digestion methods on total phosphorus determination in certified powder of Cabbage (GBW 08504) by Ascorbic acid/Molybdate Method.

| Catalyst Mixture | | Total Phosphorus Content (mg/g) | | | |
|---|---|---|---|---|---|
| Salt/Catalyst weight | (g) | Standard Kjeldahl | | Salicylic Modification | |
| $Na_2SO_4/CuSO_4$ | 1.0 | 3.89 a[1] | ***[2] | 3.88 a[1] | ***[2] |
| $Na_2SO_4/CuSO_4$ | 2.5 | 3.86 a | *** | 3.91 a | * |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 3.91 a | *** | 3.85 a | ** |

1 Figures with the same letter following are not significantly different at 5 % level using a Fisher LSD Test.

2 Values significantly different from the certified value ( 3.4 mg/g ) using a t test are indicated as follows * 5% level, ** 1% level, *** 0.01% level.

Table 3.19 Effect of catalyst mixture and digestion methods on total phosphorus determination in certified powder of Cabbage (GBW 08504) by Molybdate/Metavanadate Method.

| Catalyst Mixture | | Total Phosphorus Content (mg/g) | |
|---|---|---|---|
| Salt/Catalyst weight | (g) | Standard Kjeldahl | Salicylic Modification |
| $Na_2SO_4/CuSO_4$ | 1.0 | 3.95 a[1] ***[2] | 4.02 a *** |
| $Na_2SO_4/CuSO_4$ | 2.5 | 3.97 a *** | 3.75 a * |
| $K_2SO_4/CuSO_4/Se$ | 2.5 | 3.97 a *** | 3.96 a ** |

1 Figures with the same letter following are not significantly different at 5 % level using a Fisher LSD Test.

2 Values significantly different from the certified value ( 3.4 mg/g ) using a t test are indicated as follows * 5% level, ** 1% level, *** 0.01% level.

The measured values were significantly higher than the certified value in both plant materials by both methods of digestion and by both techniques of final determination. The reasons for the higher recoveries of phosporus are described in section 3.3.4.

### 3.3.2.2 Comparison of Ascorbic acid/ Molybdate and Molybdate/ Metavanadate techniques

The Ascorbic acid/ Molybdate method of phosphorus determination in which ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid (phosphomolybdic acid) that is reduced to an intensely coloured molybdenum blue by ascorbic acid was compared with the Molybdate/Metavanadate method of phosphorus determination in which orthophosphate and, ammonium molybdate react under acid conditions to from a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The concentration of P in solution is measured as the blue or yellow colour on the Technicon Autoanalyzer. The details of reagents and the procedure are given in Chapter 2 section 2.5. The Kjeldahl digests of the certified powders of hay and cabbage were analysed by these two methods.

Table 3.20 shows the means and standard deviations of the measured values of phosphorus in hay and cabbage by the Ascorbic acid/ Molybdate method and the Molybdate/ Metavanadate method. The means of the measured values of phosphorus of hay and cabbage by both analytical methods were significantly higher ($P< 5\%$) than the certified values. Statisticaly there is also a significant difference between the two analytical techniques. The Ascorbic acid/ Molybdate method measured values are closer to the certified values than the Molybdate/ Metavanadate method. The standard deviation of the Ascorbic acid /Molybdate

method for the cabbage powder was lower than for the Molybdate/ Metavanadate method.

Table 3.20 Comparison of Ascorbic acid/Molybdate method and Molybdate/Metavanadate method.

| Sample | Ascorbic acid | | Metavanadate. | |
|---|---|---|---|---|
| | Means ..(mg P /kg).. | Sd | Means ..( mg P /kg).. | Sd |
| Hay | 2.60 | 0.07 | 2.72 | 0.07 |
| Cabbage | 3.88 | 0.09 | 3.94 | 0.18 |

Van Lierop (1976) modified the basic Kjeldahl method (0.5 g catalyst/ml of acid, 100:10:1 $Na_2SO_4$, $CuSO_4$, Se) by various additions of hydrogen peroxide and perchloric acid to aid clearing and digestion. He obtained good recovery of K Ca Mg and P for all wet digestions. Phosphorus could not be measured by the ascorbic acid-molybdate method where Cu and Se were used due to precipitation of the metals. So the high values of total phosphorus may be due to the presence of copper and selenium as catalysts in the digest. However, no precipitation was observed with any catalyst mixture in this study.

Thomas et al. (1967) reported comparisons of the dry ashed vanadate and the sulphuric acid peroxide ashed autoanalyzer method. Their results show that vanadate method values for phosphorus are higher than ascorbic acid. Due to different methods of digestion as well as analytical technique it is not clear whether the higher values of

phosphorus are due to digestion method or analytical technique.

### 3.3.3. Potassium

#### 3.3.3.1 Effect of catalyst mixture and digestion method on potassium recovery from certified plant powders.

Tables 3.21 and 3.22 show the effects of catalyst mixture and digestion method on the determination of potassium in hay and cabbage powders respectively. These tables show two catalysts mixtures only, as determination of potassium in the treatment using Kjeltabs was not possible as they contain potassium sulphate. For both plant materials significantly higher amounts of potassium were recovered by the treatment containing 1 g of catalyst mixture compared to 2.5 g catalyst mixture by both methods of digestions. Higher potassium was measured with the salicylic acid modification method compared to the standard method for hay while for cabbage the same amount of potassium was measured with the 1 g catalyst in the standard digestion method and 2.5 g catalyst with the salicylic acid modification method.

Table 3.21 Effect of catalyst mixture and digestion methods on total potassium determination in certified powder of Hay (CRM 129).

| Catalyst Mixture | | Total Potassium Content (mg/g) | | | |
|---|---|---|---|---|---|
| Salt/Catalyst | weight (g) | Standard Kjeldahl | | Salicylic Modification | |
| $Na_2SO_4/CuSO_4$ | 1.0 | 33.3 a[1] | *[2] | 36.4 c | ** |
| $Na_2SO_4/CuSO_4$ | 2.5 | 32.7 a | * | 34.7 b | NS |

1 Figures with the same letter following are not significantly different at 5 % level using a Fisher LSD Test.

2 Values significantly different from the certified value ( 33.8 mg/g ) using a t test are indicated as follows * 5% level, ** 1% level and NS indicate value is nonsignificant from certified value.

Table 3.22 Effect of catalyst mixture and digestion methods on total potassium in certified powder of Cabbage (GBW 08504).

| Catalyst Mixture | | Total Potassium Content (mg/g) | |
|---|---|---|---|
| Salt/Catalyst weight | (g) | Standard Kjeldahl | Salicylic Modification |
| $Na_2SO_4/CuSO_4$ | 1.0 | 14.2 b[1] *[2] | 15.4 c ** |
| $Na_2SO_4/CuSO_4$ | 2.5 | 13.6 a ** | 14.2 b * |

1 Figures with the same letter following are not significantly different at 5 % level using a Fisher LSD Test.

2 Values significantly different from the certified value ( 14.5 mg/g ) using a t test are indicated as follows * 5% level, ** 1% level.

The results were compared with the certified values for potassium. As Tables 3.21 and 3.22 show, the measured values of potassium in hay and cabbage powders were significantly lower than the certified values with both catalyst mixtures in the standard Kjeldahl method. The Salicylic acid modification digestion method shows significant differences between the two catalyst mixtures for both plant materials. For the 1 g catalyst mixture with the Salicylic acid modification method the measured values of potassium were higher than the certified values. The measured potassium in the treatment with 2.5 g of catalyst mixture used with the salicylic acid modification digestion

method was not significantly different from the certified value of hay and was low for cabbage. Alfonso et al. 1991 found that the amount of sodium sulphate and copper sulphate with Se ( 2.5 g; 5 g; 10 g /20ml sulphuric acid) and time of digestion (0.75 h; 1 h; 1.5 h and 2 h ) affected the determination of K in Kjeldahl digests of plant material. They reported significantly lower K in treatments containing 2.5 g catalyst during 0.75 h digestion of plant material. Van Lierop (1976) recovered significantly higher K with a conventional Kjeldahl digestion method than with a dry ashing method. Smith (1979) estimated K content in three plant materials by three methods of digestion ( mixed acid digestion; peroxide procedure; and dry ashing). He reported that the wet digestion methods recovered similar amounts of potassium but the dry ashing method recoverd a smaller amount.

### 3.3.4 Multielement Use of Kjeldahl Digestion Method

The determination of nitrogen, phosphorus and potassium are frequently required on the same plant materials for plant nutritional studies. It is thus desirable that analyses be made on a single digest to save time, labour and cost of analyses.

Several digestion procedures have been described which allow the determination of nutrients on the same digestion solution. The most popular methods of digestion for multielement analyses are:-

i) The dry ashing method of Ward and Johnston (1960) and Varley (1966)

ii) The mixed acid ( $HNO_3$-$HClO_4$-$H_2SO_4$ ) method of Jackson (1958)

iii) The Hydrogen peroxide ( $H_2O_2$-$H_2SO_4$ ) method of Lindner (1944) and Thomas (1967)

iv) The $H_2SO_4$-Se method of O' Neill and Webb (1970)

v) The $H_2SO_4$,$H_2O_2$ $Li_2SO_4$ and Se method of Parkinson and Allen (1975) and

vi) the Kjeldahl method of digestion

Van Lierop (1976) conducted a study comparing the efficiency of several digestion procedures based on the conventional Kjeldahl digestion procedure. The digest contained 0.5 g sodium sulphate salt per ml of sulphuric acid, with various additions of hydrogen peroxide or perchloric acid to aid clearing and digestion for multielement analyses. He concluded that a wet-digestion technique using hydrogen peroxide and perchloric acid as oxidative agent in sulphuric acid medium was the most advantageous among the dry ashing and conventional Kjeldahl procedures for multipurpose analyses. But he admitted that the amount of ammonium recovered by the suggested method was lower than by the Kjeldahl method. Similar amounts of phosphorus were recovered by all digestion procedures. However, he could not measure phosphorus by the Kjeldahl procedure method because of selenium precipitation. Potassium, calcium and magnesium recoveries were less by the dry ashing method than with the Kjeldahl and hydrogen

peroxide methods. Smith (1979) compared peroxidation digestion ($H_2SO_4$-$H_2O_2$-$Li_2SO_4$-Se ), the mixed acid digestion ( $HNO_3$-$H_2SO_4$-$HClO_4$) and dry ashing procedures and reported that the mixed acid digestion method generally yielded higher Ca, Mg, and P values than dry ashing and peroxidation. The K contents of plant tissue estimated by both wet digestion techniques were not significantly different but were lower by the dry ashing method. Novosamsky (1983) determined N, P, K, Ca and Mg in plant material using a mixture of sulphuric acid and hydrogen peroxide, or a sulphuric acid-selenium, salicylic acid and hydrogen peroxide method. The measured values of all the elements were higher in the latter method.

Alfonso (1991) determined N, P, and K in sugar cane leaf by the Kjeldahl method using all combinations of 3 different amounts of catalyst mixture of sodium sulphate ( 2.5 g; 5 g and 10 g ) and four digestion times (0.75 h; 1 h; 1.5 h and 2 h ). He concluded that the amount of salt catalyst mixture has influenced the recovery of nitrogen and potassium, while the period of digestion affected recovery of nitrogen, phosphorus and potassium.

Tables 3.4, 3.6 of nitrogen; 3.16 to 3.19 of phosphorus; and 3.21 and 3.22 of potassium indicate that the use of 1 g of sodium sulphate catalyst in the standard Kjeldahl method of digestion recovered low amounts of all the elements. The salicylic acid modification method with 1 g of catalyst measured higher amounts of P and K than the certified values.

These previous investigations show that dry ashing and sulphuric acid and peroxide methods recover low amounts of nutrients. The mixed acid method recovers low amounts of ammonium nitrogen. The Kjeldahl digestion method is influenced by the time and catalyst mixture quantity. The use of selenium as catalyst can cause interference or nitrogen losses. In addition the results are not compared with the certified values for plant material or other reference materials. All the published papers show comparison of the different methods only by comparing the measured values.

## 3.4 CONCLUSIONS

The objectives of this study were to determine nitrogen, phosphorus and potassium in certified reference plant material and total nitrogen in soil samples using the three salt catalyst mixtures with the standard Kjeldahl digestion method and the salicylic modification digestion method and where possible to compare the results with certified values. A second objective was the comparison of steam distillation and the Technicon Auto-analyzer for the analysis of ammonium nitrogen. The third objective was the comparison of two colorimetric methods for determining phosphorus by the Ascorbic acid/Molybdate method and Molybdate/Metavanadate method on the Technicon Auto-Analyser.

The results in this chapter showed that the recovered values of total nitrogen and potassium but not phosphorus from certified plant materials and total nitrogen of soils are affected by the method of digestion and the catalyst mixture.

The 1 g of catalyst mixture recovered less nitrogen than the 2.5 g salt/catalyst in the standard Kjeldahl method due to the lower temperature and incomplete digestion in both plant and soil samples.

The 2.5 g catalyst mixture partially recovered nitrate in the standard Kjeldahl method and in the salicylic acid modification failed to recover all of the nitrate in certified plant powders. The nitrate content of the soil samples was not of sufficient magnitude to influence the total nitrogen values.

Use of 2.5 g catalyst mixture of sodium sulphate/ copper sulphate mixture and 2.5 g potassium sulphate/copper sulphate/ selenium mixture appears to promote nitrogen losses in the salicylic acid modification method but not in the standard Kjeldahl method of digestion for soil samples.

Little effect of catalyst mixture and digestion method was found on the recovery of phosphorus. No significant effect of catalyst composition sodium sulphate and potassium sulphate was found on phosphorus determination in plant material.

The 1 g of catalyst mixture recovered more potassium than 2.5 g of catalyst mixture in both methods of digestion for plant materials. The salicylic acid modification digestion method recovered more potassium than the standard Kjeldahl digestion method.

The steam distillation and the Technicon Auto-Analyzer techniques measured similar amounts of ammonium nitrogen. No interference effect caused by the selenium and copper was observed in the colorimetric determination of nitrogen by the Technicon Auto-Analyzer. The steam distillation method is also free from interferences. However, the Technicon Auto-Analyser method is easier, more rapid and less laborious.

The amount of phosphorus measured by the Ascorbic acid/Molybdate method was more accurate than by the Molybdate/Metavanadate method on the Technicon Auto-Analyser. No interference effects caused by selenium and copper were observed in the colorimetric determination of phosphorus by the Ascorbic acid/molybdate method or the

Molybdate/metavanadate method on the Technicon Auto-Analyzer.

The standard Kjeldahl digestion method using 2.5 g of sodium sulphate/ copper sulphate salt catalyst mixture in 5 ml of sulphuric acid, the Technicon Auto-Analyzer method for ammonium nitrogen determination and the Ascorbic acid/Molybdate Method of phosphorus determination were found to be suitable analytical techniques.

# CHAPTER 4

# NITRIFICATION

## 4.1 INTRODUCTION

Fertilization practices are often a reflection of the weather conditions that are likely to be encountered in the area. This is particularly true with nitrogen since its behaviour in soil is greatly influenced by soil moisture content and temperature. Some general points on the limitations of climate on the use of nitrogenous fertilizer in the UK have been outlined in Chapter 1. Laboratory incubation experiments are usually well defined in terms of physical, chemical and biological parameters and are therefore considered less complex than the natural environment. They do however, provide a useful model for predicting nitrogen behaviour under field conditions. In the past Anderson et al. (1969), Myers (1974) and Seifert (1980) used air dried soil samples to see the effect of temperature. Schmidt (1982) recommended freshly collected soil and minimum disturbance for determining nitrifying activities in soils. However, some researchers are still using air dried soil samples in nitrification experiments such as Yousaf (1985) and McCarty and Bremner (1989) who have used air dried samples for aerobic incubation for nitrification and mineralisation studies. Addiscott (1983), Flowers and O'Callaghan (1982), Shah (1988) and Mazumdar (1992) have used freshly collected soil samples for incubation in their studies of nitrification and mineralisation. Khan (1987) compared the effect of fresh

and air dried samples on soil nitrogen mineralisation. This short review of the literature shows that there is no single paper which directly compares the effect of fresh and air dried soil on nitrification with applied ammonium sulphate.

Anderson (1969) studied nitrification in air dried soils at 6 °C and 32 °C. Seifert (1969) measured nitrification in an air dried and remoistened soil at 5-25 °C. Shinnawi (1972) in Egypt measured the effect of air drying on nitrification. Stanford and Smith (1972 ) studied net mineralisation of nitrogen in 39 air dried soil samples by incubating them at 35 °C over a 30 week period. Myers (1974) applied 100 mg N $kg^{-1}$ to air dried soils for nitrification at 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C, 50 °C and 60 °C. Tabatabai and Alkhafaji (1980) incubated field moist samples at 20 and 30 °C for 25 weeks and studied mineral nitrogen and sulphur content every 2 weeks. Malhi & Mcgill (1982) used three air dried soils for measuring nitrification at -4 °C, 4 °C, 10 °C, 20 °C, 30 °C and 40 °C. Sahrawat (1982) compared the nitrogen mineralized by air dried samples during anaerobic incubation with that of chemical soil tests. Richter et al. (1982) studied nitrogen mineralisation in air dried soil at 35 °C. Reddy (1982) used fresh samples during incubation experiments to study the effect of fluctuating seasonal temperature on the release of inorganic nitrogen. Addiscott (1983) incubated moist soil at 5 °C, 10 °C, 15 °C, 20 °C and 25 °C to measure kinetics in nitrification. Flowers and O'Callaghan (1983) incubated fresh soil samples at 5 °C, 10

°C and 15 °C and moisture contents for the study of nitrification and mineralisation. Magdoff et al. (1983) also incubated fresh samples under aerobic conditions at 25 °C for 17 weeks and determined mineral nitrogen content at 2 week intervals. Farooqi et al. (1983) studied nitrogen mineralisation using air dried soil samples at a constant temperature of 35°C. Griffin and Laine (1983) incubated air dry samples at 35°C for 40 weeks and the mineral nitrogen was determined after different intervals of time. Hussain et al. (1984) and Darah et al. (1985) also used air dry soils during aerobic experiments. Nordmeyer and Richter (1985) incubated soils in the fresh and air dried state at different temperatures (20 and 25°C) and compared the nitrogen mineralisation after 16 weeks of incubation. Macduff and White (1985) studied nitrogen mineralisation and nitrification in field moist clay soils in laboratory incubation experiments at 4 °C, 10 °C and 20 °C. Yousaf (1985) incubated soil for nitrification of applied fertilizer in air dried soils at 10-20 °C. Khan (1987) compared the effect of air dried and fresh soils on mineralisation at 20 °C. Shah (1988) incubated fresh coal mine soils in a study of transformation of nitrogen and its availability to plants at 20 °C. Mazumdar (1992) also incubated two fresh soil to see the inhibition of nitrification by pesticides in two soils at 20 °C.

The above review of the literature indicates that methods of incubation of soils are not uniform. There is a lack of information regarding comparisons of the rates of nitrification in fresh and air dried soils, and there

are few studies showing the effects of low temperature on nitrification with applied ammonium sulphate on freshly collected soil samples. None of these studies show the effect of temperature at 0 °C with applied ammonium sulphate or repeated applications of ammonium sulphate on nitrification.

In the present study it was planned to compare rates of nitrification in fresh and air dried soils and to examine the effect of low temperature and two applications of fertilizer on the nitrification of applied ammonium sulphate in soils of different organic matter content, textural class and which were collected from different climatic regions of Britains. At the same time short term studies were made of the rate of mineralisation.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Materials

Five soil samples were collected, two from England (Midelney and Freckenham) and three (Darvel, Dreghorn and Darlieth) from Scotland. The soil sampling sites and descriptions for the Midelney and Dreghorn series soil are given in Chapter 3 section 3.2.1. The soil sampling sites for Darvel, Freckenham and Darlieth series soils with a brief description is given below. Soils were assigned to soil series using the Soil Memoirs and Soil Maps for each area. Where the area had not been mapped, Soil Memoirs for an adjacent area were used. (Mitchell and Jarvis, 1956; Grant et al., 1962; Seale, 1975; Ragg et al. 1976).

Darvel series soil

The site is located at Westerton farm, Lennoxtown, Scotland. The grid reference is NS 635773. The soil is cultivated under mixed cropping. 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.

Freckenham series soil

The soil series occurs on flat or gently undulating land. The soil is mainly developed in non-calcareous water laid sands and gravels. The soil is under

cultivation. The Freckenham soils are freely or excessively drained sands and loamy sands at least 1 metre deep.

Darlieth series soil

The site is located at Milngavie, Glasgow, Scotland. Grid reference is NS 553736. The soil is used for cultivation as a garden. The soil belongs to the Darleith Association which is developed on till derived from Carboniferous age igneous rocks (Basalt). The soil Series is Darleith which has been classed as a freely drained brown forest soil.

Table 4.3 Physical properties of soils (Khan,1987 & Mazumder,1992).

| | Darvel | Dreghorn | Midelney | Freckenham | Darlieth |
|---|---|---|---|---|---|
| LOI (%) | 9.1 | 5.6 | 16.2 | 4.0 | 23.3 |
| pH water | 7.4 | 6.5 | 6.9 | 6.7 | 5.9 |
| Sand (%) | 33.5 | 87.9 | 17.8 | 84.8 | 39.3 |
| Silt (%) | 22.0 | 10.9 | 40.3 | 8.8 | 19.2 |
| Clay (%) | 42.4 | 1.2 | 42.3 | 5.6 | 41.5 |
| Texture class | clay | sand | clay | loam/sand | silt/clay |

1) Sand >53 μm. silt >2 μm clay <2 μm

4.2.2 Methods

4.2.2.1 Sampling and sample pretreatments

The samples were taken from the cultivated depth (0-30 cm ) of agricultural or garden soils. The freshly collected samples were brought to the laboratory as soon as possible. The samples were spread out on clean plastic sheets for drying. The wet samples were partially air dried in the laboratory just sufficiently to pass through a 4 mm sieve easily. Then a portion of each soil was air dried at the laboratory temperature for two days. The fresh subsamples were stored in the cold room as on air dried subsample at room temperature in labelled plastics bags until used in the experiment. The samples were tested for moisture content, -0.5 Bar moisture content, pH and initial level of ammonium, nitrite and nitrate.

4.2.2.2 Routine Methods

The percent moisture content and -0.5 Bar moisture content were measured as per sections 2.9 and 2.12. The pH measurements in 2.5:1, water:soil solution were measured as described in Chapter 2, section 2.13.

4.2.2.3 Incubation procedure

A sample of soil equivalent to 50 g (oven dry weight basis) was weighed into an 500 ml glass bottle which was left open to permit aeration. The sample was treated with 100 mg N $g^{-1}$ of soil by adding 1 ml of ammonium

sulphate solution containing 5000 mg $NH_4$-N $l^{-1}$. Each soil sample was mixed thoroughly using a spatula 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 glass 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 day zero, each 500 ml bottle was then placed in a large plastic tub. This was lined with damp 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 lids of the tubs were tightly sealed to prevent water loss. The plastic tubs were large enough to ensure that there was sufficient oxygen for the incubation period. The tubs were kept in incubators at the required temperature. The change in the ammonium, nitrate and nitrite nitrogen were measured at intervals. In order to determine rates of nitrification and mineralisation, subsamples of each soil were taken from the bottle at several time intervals. The duration of the time intervals was varied with the incubation temperatures and soil types. The plastic tubs were allowed to stand open for 10-15 minutes to replenish the air at the end of each interval. The subsamples taken were extracted for measuring the concentration of ammonium, nitrate and nitrite.

The soil samples were incubated until complete conversion of ammonium into nitrate.

4.2.2.4 Extraction of Inorganic Nitrogen

Ammonium-N, nitrate-N and nitrite-N were extracted (section 2.7) by shaking 2.5 g ( oven dry basis) of incubated soil with 50 ml of 0.5 M potassium sulphate solution for 2 hours at 2 °C. The suspensions were filtered using acid washed Whatman filter papers No. 1 (section 2.7.2)

4.2.2.5 Determination of Inorganic Nitrogen

Ammonium

Determination of the concentration of ammonium nitrogen in the extracts was carried out by the Technicon Autoanalyzer system as described in section 2.2.2

In addition to the standard stock solution ( 1000 N mg $l^{-1}$ ) and reagents for the ammonium manifold as in Chapter 2 section 2.2.2, 0 and 5 mg N $l^{-1}$ for ammonium, working standards were prepared in potassium sulphate solution .

The filtered solutions were analysed directly on the Technicon Autoanalyzer II system at a rate of fifty samples per hour.

Nitrate and Nitrite

The concentration of nitrate and nitrite in the soil extracts was measured by the Technicon Autoanalyzer system by the method in Chapter 2 sections 2.4 and 2.5.

In addition to the standard stock solution ( 1000 mg N $l^{-1}$ ) and reagents for the determination of nitrate and nitrite as described in Chapter 2 section 2.4 and 2.5, the following working standards in potassium sulphate solution were made up 0, 1, 2, 3, 4, and 5 mg N $l^{-1}$ and 0, 2, 4, 8, 12 mg N $l^{-1}$ for nitrate and 0 and 1 mg N $l^{-1}$ for nitrite determination.

The filtered solutions were analysed directly on the Technicon Autoanalyzer II system at a rate of fifty samples per hour.

4.2.3 Experimental Design

All the soils were incubated at -0.5 Bar moisture potential and treated with 100 mg N $g^{-1}$ of soil. Three experiments with four replications of each soil were carried out. Details of soils, incubation temperature, and the objective of each experiment are given below :-

1. Effect of air drying soils on nitrification

The fresh and dried and remoistened subsamples of Darvel, Dreghorn, Midelney, Darlieth and Freckenham were incubated at 15 °C for measuring the effect of air drying.

2. Effect of temperature on nitrification

The fresh soil samples of Darvel, Freckenham, Midelney and Darlieth were incubated at 0, 5, 10, 15 and 20 °C.

3. Effect of two applications of ammonium-N on nitrification

The fresh soil samples of Darvel, Darlieth Freckenham and Midelney were treated with 100 mg $NH_4$ N $g^{-1}$ of soil on oven dried basis and incubated at 15 °C. After conversion of all the ammonium to nitrate, a second application of 100 mg $NH_4$ N $g^{-1}$ of soil was made and incubation continued until conversion of all of the second dose of applied ammonium to nitrate.

### 4.2.4 Statistical Analysis.

The measured values of ammonium-N nitrate-N and total inorganic-N were plotted on graphs by using the Cricket Graph package (version 1.3).

The Minitab package (version 7.2) was used for calculating rates of ammonium disappearance, nitrate formation and mineralisation. The rates were calculated by regressing the measured values on time.

To test the significance of the effect of air drying on the rates of ammonium disappearance, nitrate formation and mineralisation of nitrogen, the measured values of four replications for fresh and air dried soils were pooled into two regression lines. A t-test was used to test the difference between the two regression slopes. Similarly the effect of the first and second applications on the rate of ammonium disappearance was tested using a t test.

## 4.3 RESULTS AND DISCUSSIONS

The measured quantities of ammonium and nitrate were added to give total inorganic nitrogen and graphs were plotted against time. Regression lines were fitted to the linear part of the curves.

The analyses showed the accumulation of $NO_2$ -N was smaller than 2 mg $kg^{-1}$ in the case of Darvel, Dreghorn, Darlieth and Freckenham soils but was up to 8 mg $kg^{-1}$ in the case of Midelney soil. So $NO_2$ -N is not plotted on the figures.

### 4.3.1 Effect of air drying on Nitrification and mineralisation.

The effect of previous air drying on the nitrification of added ammonium sulphate during incubation at -0.5 Bar soil moisture potential and 15 °C is shown in Figures 4.1 to 4.10. Although the nitrification period of Darvel, Dreghorn, Midelney, Darleith and Freckenham soils was quite different, the figures of all the soils were made on the same scales for easy comparison. Typical data for single incubations are shown in the figures.

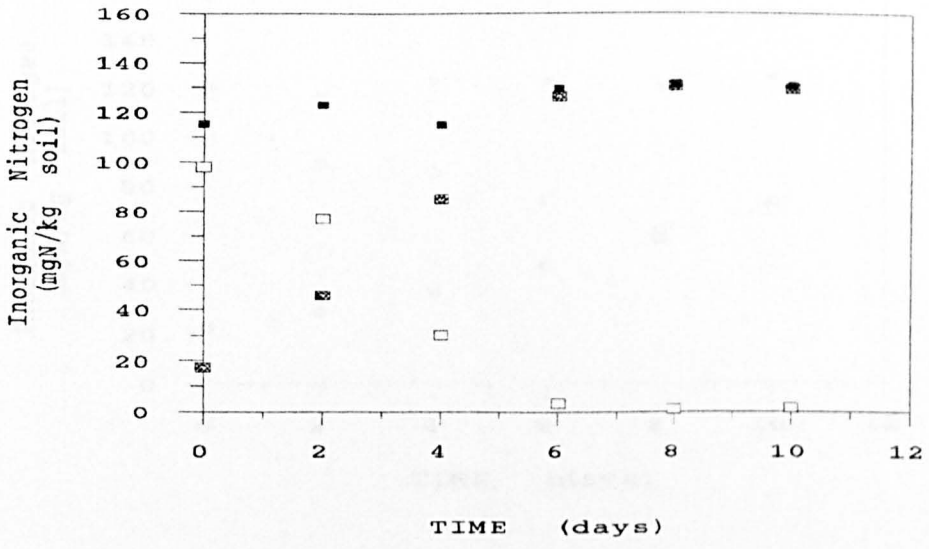



Fig 4.1 Nitrification in fresh Darvel soil

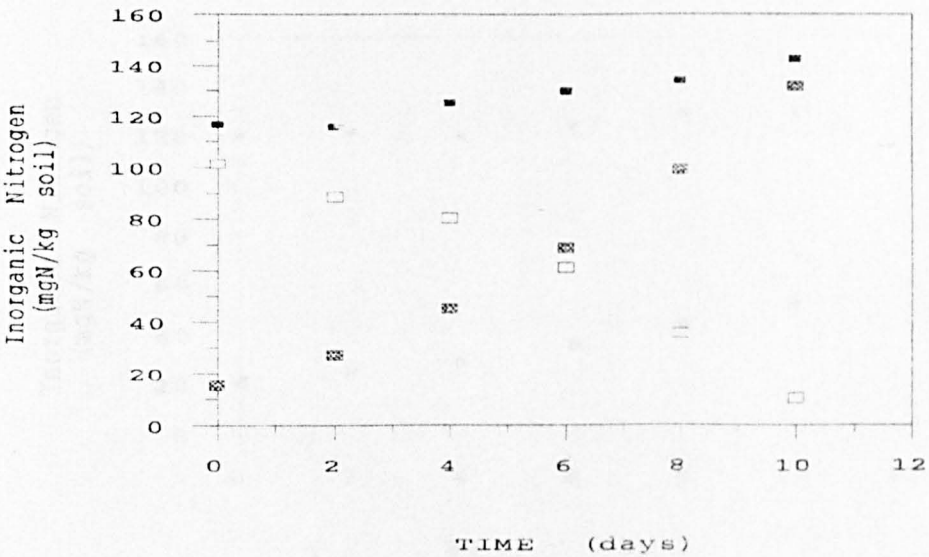



Fig 4.2 Nitrification in air dried Darvel soil

□ Ammonium-N
▩ Nitrate-N
▪ Total inorganic-N

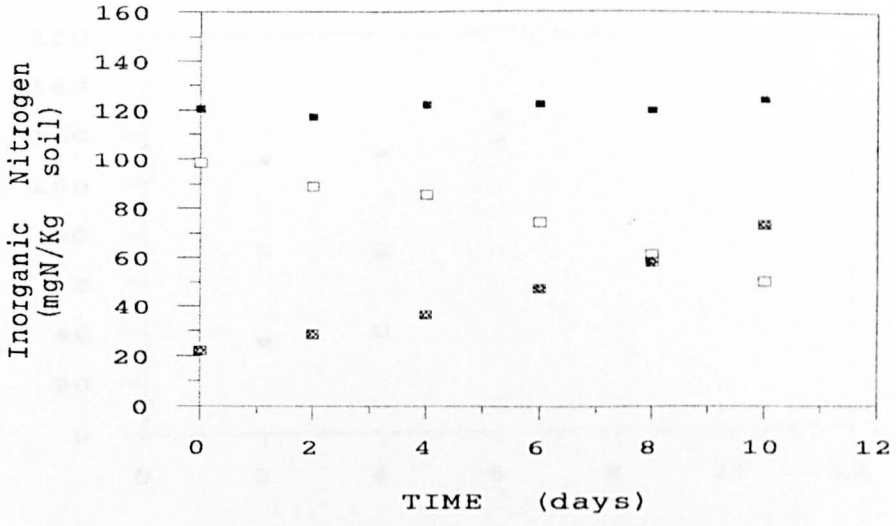



Fig 4.3 Nitrification in fresh Dreghorn soil

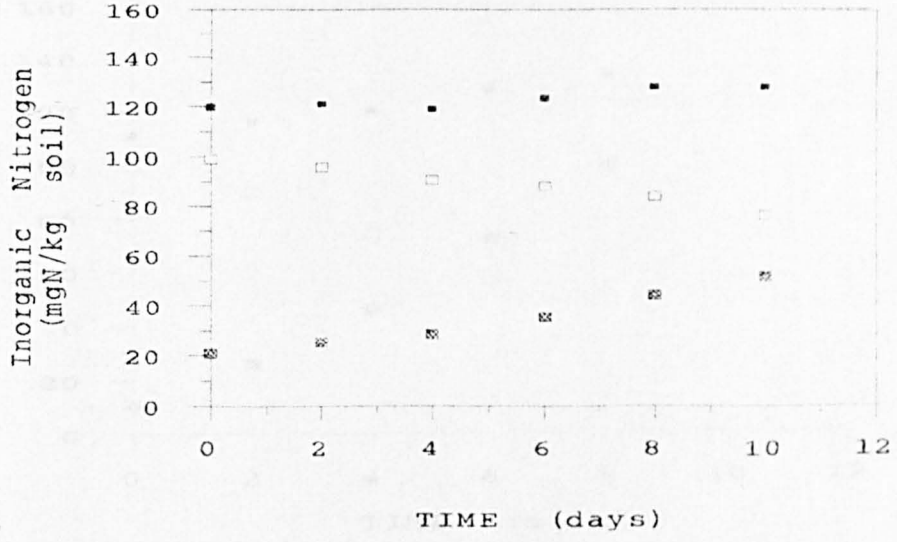



Fig 4.4 Nitrification in air dried Dreghorn soil

□ Ammonium-N
▩ Nitrate-N
■ Total inorganic-N

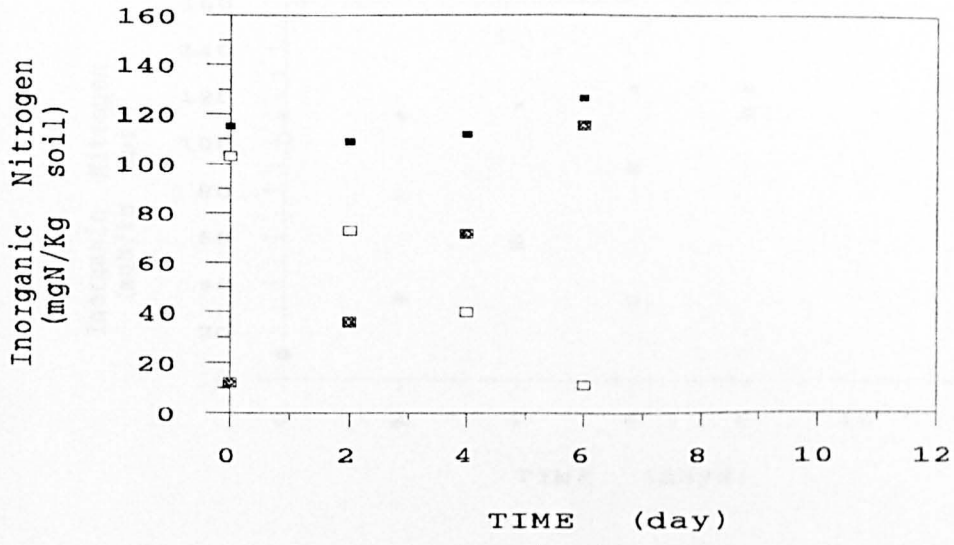



Fig 4.5 Nitrification in fresh Darlieth soil

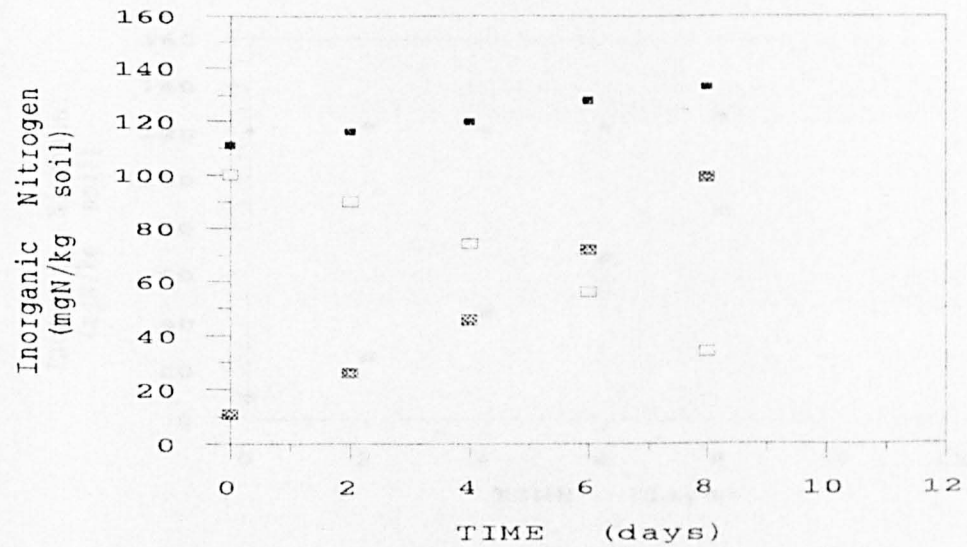



Fig 4.6 Nitrification in air dried Darlieth soil

□ Ammonium-N
▩ Nitrate-N
■ Total inorganic-N

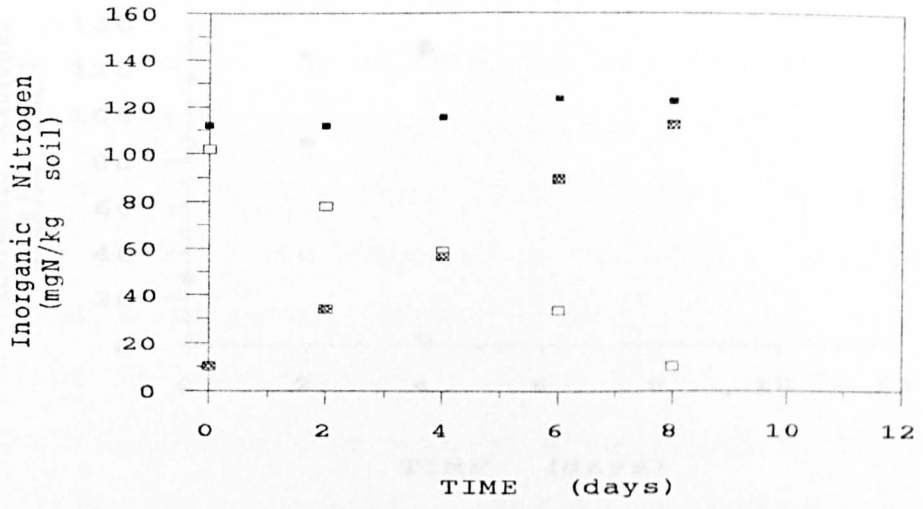



Fig 4.7 Nitrification in fresh Freckenham soil

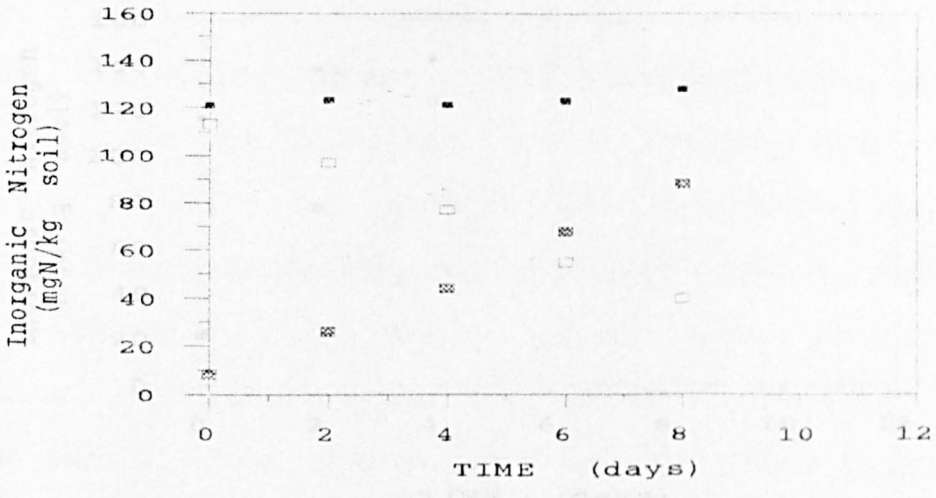
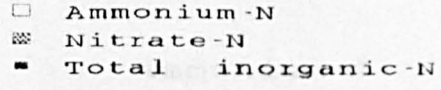



Fig 4.8 Nitrification in air dried Frechenham soil



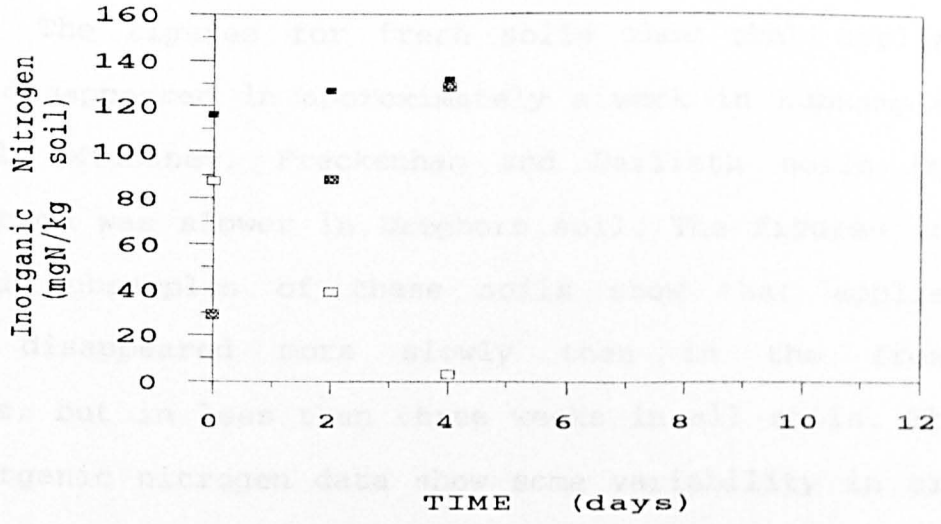



Fig 4.9 Nitrification in fresh Midelney soil

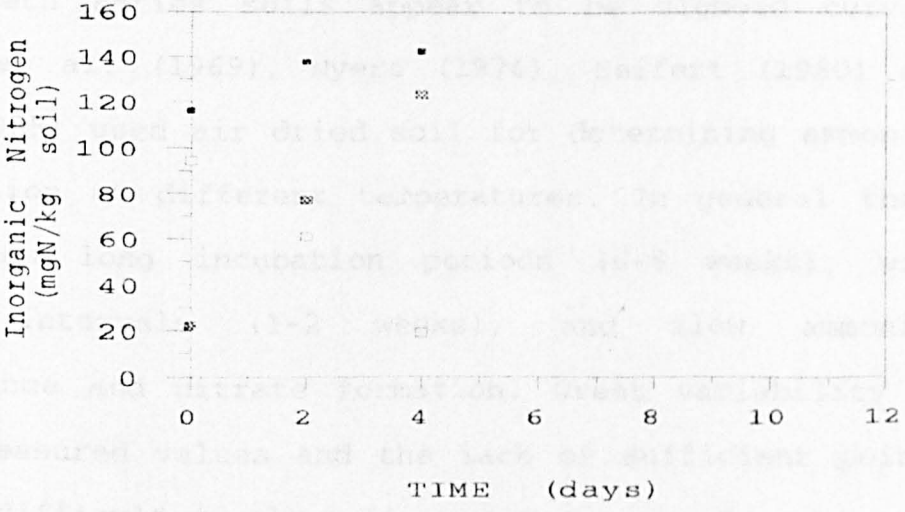



Fig 4.10 Nitrification in air dried Midelney Soil

□ Ammonium-N
▩ Nitrate-N
■ Total inorganic-N

The figures for fresh soils show that applied ammonium disappeared in approximately a week in subsamples of Darvel, Midelney, Freckenham and Darlieth soils but nitrification was slower in Dreghorn soil. The figures for air dried subsamples of these soils show that applied ammonium disappeared more slowly than in the fresh subsamples, but in less than three weeks in all soils. The total inorganic nitrogen data show some variability in all soils but there was a general upward trend indicating a small amount of mineralisation. The Midelney soil ammonium disappeared faster than expected, and only three points were found on the line. Figures 4.2 and 4.6 for the air dried Darvel and Darlieth soil series show a slight lag period in ammonium disappearance and nitrate formation. The nitrate formation curves for the air dried soils of Darvel and Darlieth series soils appear to be sigmoid curves. Anderson et al. (1969), Myers (1974), Seifert (1980) and Yousaf (1985) used air dried soil for determining ammonium nitrification at different temperatures. In general their papers show long incubation periods (6-8 weeks), wide sampling intervals (1-2 weeks), and slow ammonium disappearance and nitrate formation. Great variability is seen in measured values and the lack of sufficient points makes it difficult to draw lines and to see the shape of the nitrification curves. Some papers show lines but due to wide sampling interval it is difficult to see the precise lag period or to measure rates. For example Seifert (1980) has drawn sigmoid curves with two points.

Addiscott (1983), Flowers and O'Callaghan (1982) and Mazumdar (1992) used fresh soils and Shah (1988) used fresh coal mine spoils for measuring nitrification rates. Their results show short incubation periods, more frequent sampling intervals, very low variability, and clear shapes of lines indicating no lag period. Ammonium disappearance and nitrate formation fit a straight line and zero order rate constants were calculated for the nitrification rates.

In this experiment nitrification and mineralisation rates were calculated by using linear regression to attain the best fit straight line to the linear part of the nitrate formation, ammonium disappearance and total inorganic nitrogen levels. Table 4.4 shows the means of rates of nitrification and mineralisation.

Table 4.4 Nitrification rates in fresh and air dried soils

| | Fresh ( mg N $kg^{-1}$ soil | Dry $day^{-1}$) |
|---|---|---|
| Darvel | | |
| Nitrate formation rate | 17.6 b | 13.6 a |
| Ammonium disappearance rate | 16.4 b | 12.1 a |
| Mineralisation rate | 1.9 a | 2.8 b |
| Dreghorn | | |
| Nitrate formation rate | 8.8 b | 7.4 a |
| Ammonium disappearance rate | 6.5 a | 5.9 a |
| Mineralisation rate | 1.2 a | 1.3 a |
| Midelney | | |
| Nitrate formation rate | 27.1 a | 25.5 a |
| Ammonium disappearance rate | 27.2 a | 20.6 a |
| Mineralisation rate | N.I. | 4.3 |
| Freckenham | | |
| Nitrate formation rate | 13.3 b | 10.5 a |
| Ammonium disappearance rate | 11.9 b | 8.2 a |
| Mineralisation rate | 1.4 a | 2.3 b |
| Darlieth | | |
| Nitrate formation rate | 13.0 b | 11.4 a |
| Ammonium disappearance rate | 12.2 b | 8.1 a |
| Mineralisation rate | 1.1 a | 3.3 b |

1) Data in a row with same subscript letter are not significantly different at 5% level (pooled t-test).

N.I. Nitrogen Immobilisation.

The nitrate formation rates were significantly higher in the fresh subsamples than in the air dried subsamples of Darvel, Dreghorn, Freckenham and Darlieth soils. The rates of ammonium disappearance were significantly higher in fresh Darvel, Freckenham and Darlieth subsamples compared with the air dried samples but

the differences were nonsignificant in the Dreghorn and Midelney soils. The reason for the lower rates of nitrate formation and ammonium disappearance in the air dried subsamples of Darvel, Dreghorn, Freckenham and Darlieth soils was probably the death of some of the ammonium and nitrite oxidising microorganisms. Mortenson and Duley (1931) stated that, the air drying and storage of soils affect soil properties and microorganisms, and in many cases decreased the nitrifying power. Harpsted and Brage, (1958) reported that drying and storage of soil decreased its nitrification power. Bottner (1985) reported that on air drying of soil up to 1/3 or 1/4 of its biomass was killed depending upon the soil properties, but Fraps and Sterges (1932) found that bacteria do exhibit resistance to desiccation, and a few organisms remain viable in soils kept for up to 14 to 18 years in the air dry state. Decreases in microbial biomass (by 1/6 to 1/3) after desiccation of soils have been reported often (Amato and Ladd, 1980; Sorensen, 1983; Bottner, 1985; Kieft et al. 1987). Van Gestel et al. (1991) observed that similar proportions of biomass disappeared after drying soils of contrasting texture and aggregate stability but from the same climatic region, and they suggested that adaptation of the soil biota to climatic conditions involving frequent and severe soil drying, may have had an overriding influence. Allison (1991) reported the survival of ammonia-oxidizing bacteria in air dried soil. The soils used in the present experiments were air dried at laboratory temperature for only 48 hours and stored for not more than

a week. This may not be a severe treatment of air drying for the ammonium and nitrite oxidising microorganisms. Therefore, except for the Darvel soil, no large differences in nitrification rates were observed.

The mineralisation rates were higher in the air dried subsamples than fresh subsamples of all the soils. The mineralisation rate in fresh soil was in the range of 1.1-1.9 mg N $kg^{-1}$ soil $day^{-1}$ and in air dried soils the rate was 1.3 to 4.3 mg N $kg^{-1}$ soil $day^{-1}$. The reason for the increased mineralisation rates in air dried soil is the death of a more general population of microorganisms, decomposition of organic matter and the disturbance of soil structure. Air drying and storage of soils are known to affect properties (Birch, 1958) and microorganisms (Ross & Mc Niel, 1975). Drying and remoistening of soils strongly influences microbial biomass and activity (Lund and Goksoyr, 1980; Orchard and Cook, 1983 ; Bottner, 1985 ; Kieft et al., 1987). Wetting of soil after a drying period increases nitrogen mineralisation (Jacquemin and Berlier, 1956; Seifert, 1969). Jacquemin and Berlier (1956) reported that, mineralisation occurs rapidly in the wet period following a prolonged drought or in the rainy season following a long dry spell. The mineralisation rate was found to increase with increase in soil organic matter particularly in air dried soils. Berg and Rosswall (1985) reported that the organic matter content of soil seems to affect nitrifier numbers as well as actual and potential activities. Khan (1987) compared mineralisable carbon and

nitrogen in fresh and air dried soils by incubating for 12 weeks and reported that the air dried samples were physically disturbed during the drying operation. He measured a large flush of carbon dioxide and ammonium production in the first week of incubation in air dried soil. He also found a good correlation between total C released, total nitrogen, total C and biomass in air dried soils samples.

### 4.3.2. Effect of Temperature on nitrification and mineralisation.

The effect of temperature on nitrification of applied ammonium sulphate in fresh samples of Darvel, Midelney, Freckenham and Darlieth soils was studied by incubating samples of each soil at 0, 5, 10, 15 and 20 °C ( plus or minus 0.5 °C at each temperature). Figures 4.11 to 4.15 show the time on the X-axis and the concentration of ammonium, nitrate and total inorganic nitrogen on the Y-axis. The incubation periods vary with soil type and incubation temperature. However, an attempt was made to standardise the graph axis to allow easy comparisons of the figures. The figures for 20 °C and 15 °C have the same axis scales while the figures for 10 °C, 5 °C and 0 °C have to cover longer incubation periods. Typical data for the Freckenham soil from single incubations are shown in the figures.

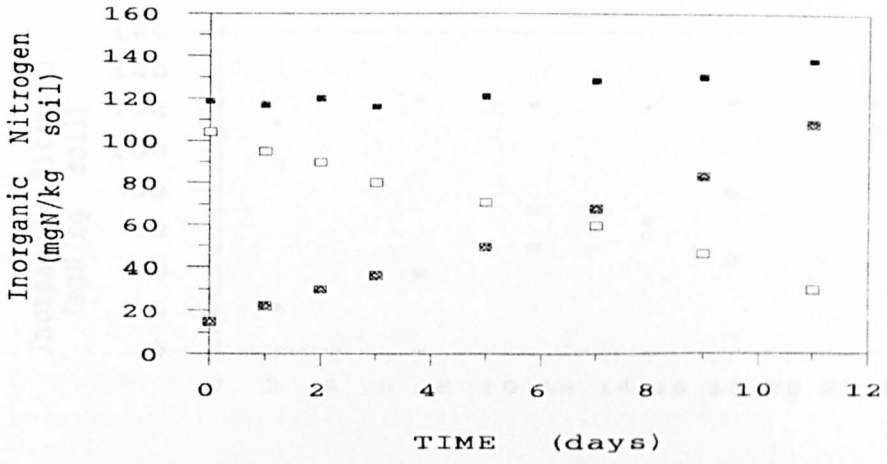



Fig 4.11 Nitrification in Freckenham soil at 20 C

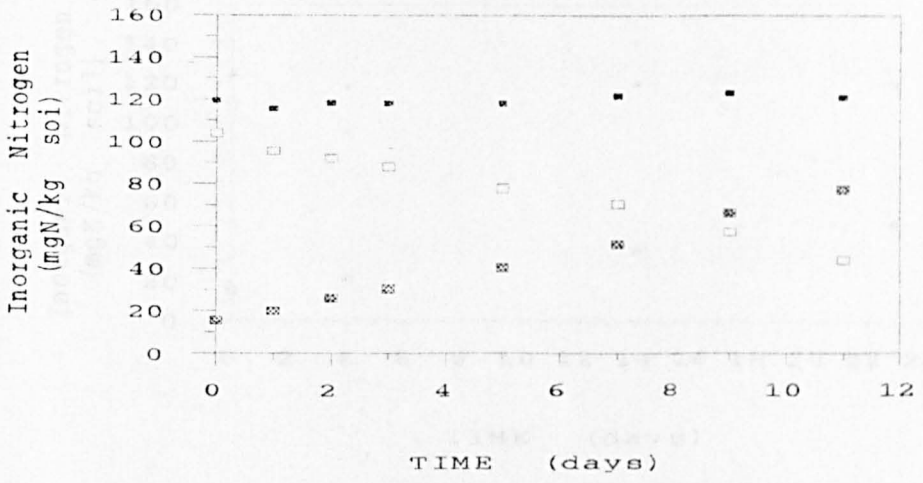



Fig 4.12 Nitrification in Freckenham soil at 15 C

□ Ammonium-N
▩ Nitrate-N
▪ Total inorganic-N

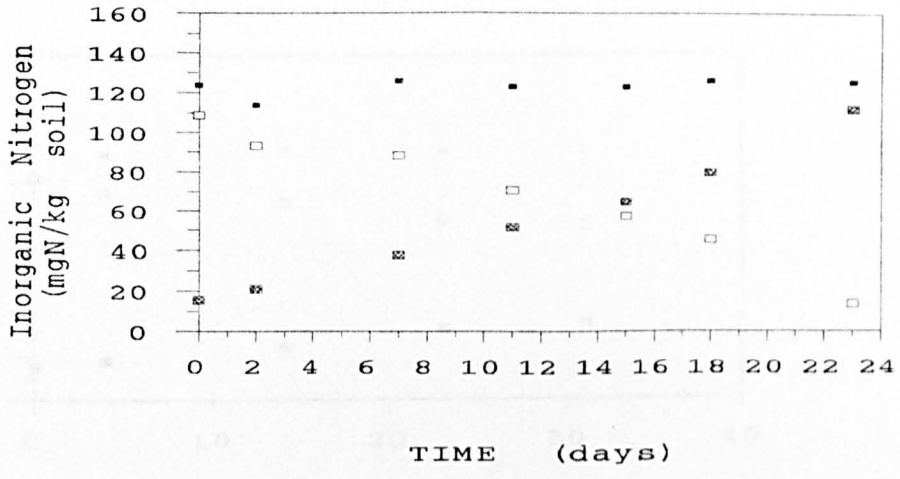



Fig 4.13 Nitrication in Freckenham soil at 10 C

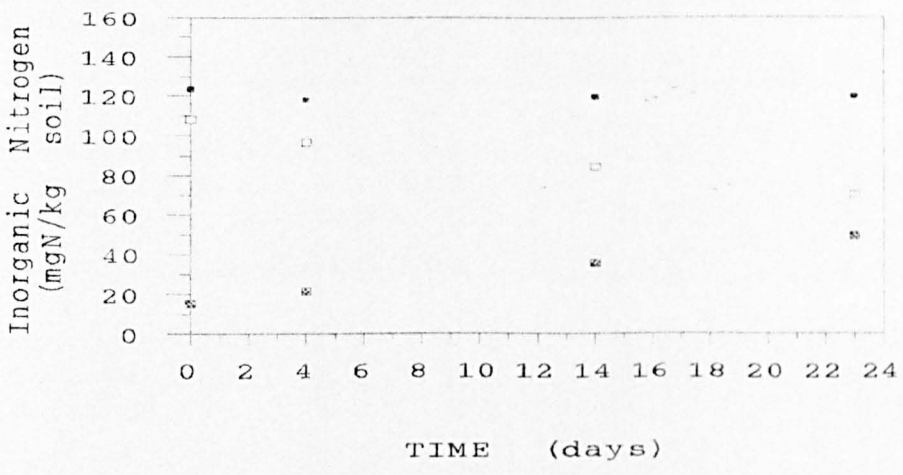



Fig 4.14 Nitrification in Freckenham soil at 5 C

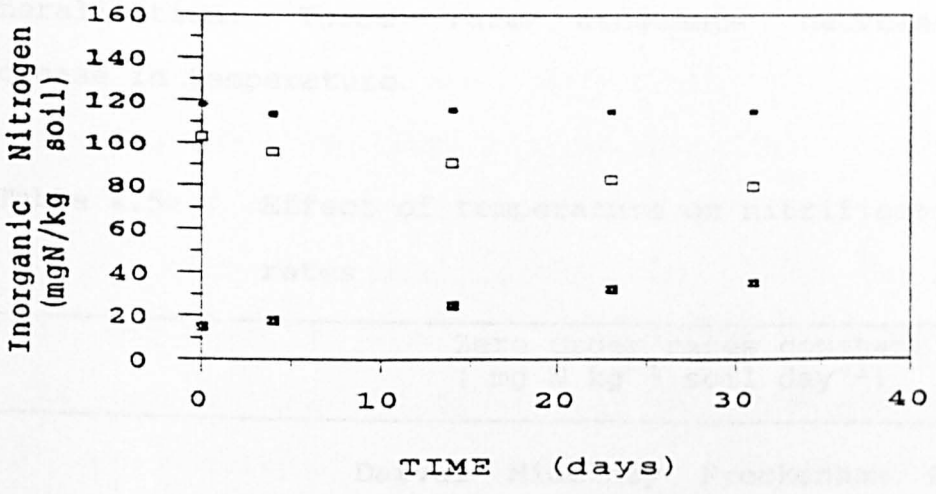



Fig 4.15 Nitrification in Freckenham soil at 0 C

□ Ammonium-N
▪ Nitrate-N
■ Total inorganic-N

Table 4.5 shows the zero order rate constants for nitrate formation, ammonium disappearance and nitrogen mineralisation. These rate constants decreased with decrease in temperature.

Table 4.5 Effect of temperature on nitrification rates

| | Zero order rates constant ( mg N $kg^{-1}$ soil $day^{-1}$) | | | |
|---|---|---|---|---|
| | Darvel | Midelney | Freckenham | Darlieth |
| 20 °C | | | | |
| Nitrate formation rate | 24.6 | 36.2 | 8.1 | 26.4 |
| Ammonium disappearance | 21.5 | 43.0 | 6.6 | 21.9 |
| Mineralisation rate | 2.9 | N.I. | 1.6 | 4.4 |
| 15 °C | | | | |
| Nitrate formation rate | 15.1 | 27.1 | 5.6 | 23.6 |
| Ammonium disappearance | 13.2 | 27.2 | 5.2 | 20.3 |
| Mineralisation rate | 2.2 | N.I. | 0.3 | 3.3 |
| 10 °C | | | | |
| Nitrate formation rate | 10.6 | 23.6 | 4.1 | 11.7 |
| Ammonium disappearance | 8.7 | 25.9 | 3.7 | 9.7 |
| Mineralisation rate | 1.9 | N.I. | 0.3 | 2.0 |
| 5 °C | | | | |
| Nitrate formation rate | 4.5 | N.D. | 1.5 | 6.5 |
| Ammonium disappearance | 4.0 | N.D. | 1.4 | 5.1 |
| Mineralisation rate | 0.8 | N.D. | 0.1 | 1.3 |
| 0 °C | | | | |
| Nitrate formation rate | 1.8 | N.D. | 0.6 | 3.7 |
| Ammonium disappearance | 1.5 | N.D. | 0.7 | 3.0 |
| Mineralisation rate | 0.3 | N.D. | 0.0 | 0.7 |

N.I. Nitrogen Immobilisation
N.D. Not Determined.

Nitrification has been reported to proceed at temperatures ranging from freezing up to 50 °C ( Myers 1975). The optimum temperature, however, as found by Sabey et al. (1959), Justice and Smith (1962), Thiagalingam and Kamebiro (1973), Kowalenko and Cameron (1976) is generally in the range from 25 to 35 °C.

Although nitrification has been investigated extensively this has been mainly concerned with the process at the temperatures found during the warm season. There have been few reports concerned with the nitrification of ammonical fertilizers at low temperatures (Anderson and Purvis 1969; Myers 1974; Tyler et al. 1958; and Yousaf 1985). These few studies all show the effect of temperature on the nitrification process in air dried soil samples. Very few studies have used fresh soil samples (Addiscott 1983 and Flowers and O'Callaghan 1983).

Therefore, in the present study fresh soil samples were used to investigate the effects of temperature. The rates of ammonium disappearance and nitrate formation were different in all soils. The ammonium disappearance rates were smaller than nitrate formation rates in Darvel, Freckenham and Darlieth soils, but larger in Midelney soil. At all temperatures the rates in the four soils remained in the order Midelney > Darlieth > Darvel > Freckenham. The rates of nitrate formation at 0 °C and 20 °C were in the range of 0.6 to 3.7 and 8.1 to 36.2 mg N $kg^{-1}$ soil $day^{-1}$ respectively. Addiscott (1983) incubated three soils to measure the kinetics and temperature relationships of mineralisation and nitrification in

Rothamsted soils at 2.5, 5, 10, 15, 20 °C. He reported that the rates of nitrification increased in the range of 0.45 to 2.54 mg N $kg^{-1}$ $day^{-1}$ at 2.5 °C to 12.7 mg N $kg^{-1}$ $day^{-1}$ at 20 °C. In the present study the soils showed higher rates than the soils used by Addiscott (1983). The lowest temperature at which Addiscott measured nitrate formation and ammonium decline rates was 2.5 °C in all three soils. The nitrification rates of two soils were markedly decreased when the temperature decreased from 5 °C to 2.5 °C. In the third soil, nitrification rate fell less steeply between 5 and 2.5 °C. In the present study the soils were incubated at temperatures ranging from 20 °C to 0 °C. The rates of nitrification were calculated for all temperatures for three out of four soils. The nitrification rates could not be calculated in one soil due to a sudden fault in the incubators in which that soil was contained.

To explain the sharpness in decrease in nitrification rates as affected by the temperature, $Q_{10}$ values were calculated from the ratio of the rate at 20 °C and 10 °C, 15 °C and 5 °C and 10 °C and 0 °C as shown in Table 4.6.

Table 4.6 $Q_{10}$ values of temperatures

| $Q_{10}$ | Darvel | Midelney | Freckenham | Darlieth |
|---|---|---|---|---|
| 20/10 °C | | | | |
| Nitrate formation rate | 2.3 | 1.5 | 1.9 | 2.2 |
| Ammonium disappearance | 2.4 | 1.6 | 1.8 | 2.2 |
| 15/5 °C | | | | |
| Nitrate formation rate | 3.3 | - | 3.7 | 3.6 |
| Ammonium disappearance | 3.3 | - | 3.7 | 3.9 |
| 10/0 °C | | | | |
| Nitrate formation rate | 5.8 | - | 6.8 | 3.2 |
| Ammonium disappearance | 5.8 | - | 5.2 | 3.2 |

The $Q_{10}$ values for nitrate formation and ammonium disappearance were in the range of 1.5 to 6.8 and 1.6 to 5.8 respectively. The $Q_{10}$ values increased with decrease in temperature. The $Q_{10}$ values above 10 °C are near two while below 10 °C are more than four. Thus a sharp decrease in the rates was measured at below 10 °C in these soils.

The interpretation of $Q_{10}$ values at low temperatures requires great care as threshold effects may have a large effect on the calculated value. Clearly a $Q_{10}$ value has no meaning if the rate falls to zero at the lower end of the temperature range. However, if only part of the active population has a temperature threshold within the temperature range used for the calculation then a large $Q_{10}$ value may result. This is more likely to occur with a mixed population than with a single species thus nitrification would be expected to be less affected than mineralisation.

To test the relationship between the rates of nitrate formation, ammonium disappearance and

mineralisation with temperature, the natural logarithm of the rates was plotted against the reciprocal of absolute temperature. A straight line was fitted to give the Arrhenius relationship by regressing the absolute temperature on Ln (rate), using the Minitab package .

$$\text{Ln (K)} = \text{Ln (A)} - B/T$$

where K is the zero order constant rates, A and B are constants and T is the absolute temperature. The B coefficient is a measure of the temperature sensitivity of the process. The fitted constants are shown in Table 4.7 for each soil.

Table 4.7 Arrhenius regressions ( Ln (K) on 1/T )

| Soil | Intercept ln(A) | Slope B coefficient (°K) | $R^{2*}$ |
|---|---|---|---|
| Darvel | | | |
| Nitrate formation | 38.2 | -10246 | 97.0 |
| Ammonium disappearance | 38.5 | -10363 | 97.1 |
| Mineralisation | 31.8 | -8935 | 91.5 |
| Freckenham | | | |
| Nitrate formation | 37.7 | -10395 | 95.0 |
| Ammonium disappearance | 33.6 | -9253 | 95.0 |
| Mineralisation | N.D. | N.D. | N.D. |
| Darlieth | | | |
| Nitrate formation | 31.7 | -8299 | 97.2 |
| Ammonium disappearance | 32.3 | -8521 | 96.6 |
| Mineralisation | 26.5 | -7317 | 98.7 |

* $R^2$ is coefficient of determination.
N.D. Not Determined

It is clear from the above table that the data fit the Arrhenius relationship well. The relationship was found to be improved by omitting the 0 °C data for the

Darvel soil and much improved by omitting the 20 °C data for the Darlieth and Freckenham soils. (See Figures 4.16 to 4.23). The data in Table 4.7 were obtained without omitting any data points. No published Arrhenius coefficients for nitrification in soils were found except those of Addiscott (1983) but he could only show a significant linear relationship with one out of three soils examined. He reported that the relationship for the other two soils was not significant because of the sharp decrease in rates between 5 °C and 2.5 °C. Omitting 2.5 °C much improved the relationships. The B coefficient values of Addiscott (1983) were -7145 °K, -6723 °K and -7634 °K for ammonium decline, nitrate formation and nitrogen mineralisation respectively. These values were not significantly different, indicating the sensitivity to temperature of nitrification and nitrogen mineralisation are equal. In the present study the B coefficient value of mineralisation for the Freckenham soil was not calculatable due to nonsignificant differences in the rates of mineralisation at the different temperatures. The B coefficient values of nitrification are higher than those Addiscott reported.

In the present study the mineralisation rates were calculated for a short incubation period with applied ammonium sulphate at 100 mg N $kg^{-1}$ soil. The calculated mineralisation rates also show an increase with increase in temperature in the following order in incubated soils: Darlieth > Darvel > Freckenham. In the Midelney soil, instead of showing mineralisation of ammonium, immobilisation was observed.

The mineralisation rates also fit well with the Arrhenius relationship as discussed above. The B coefficient values for mineralisation in the present studies agree with the values reported earlier. Tabatabai and Al-Khafaji found an average B coefficient for 12 soils of -8871 °K for nitrogen mineralisation and Addiscott (1983) -7000 °K for all three processes.

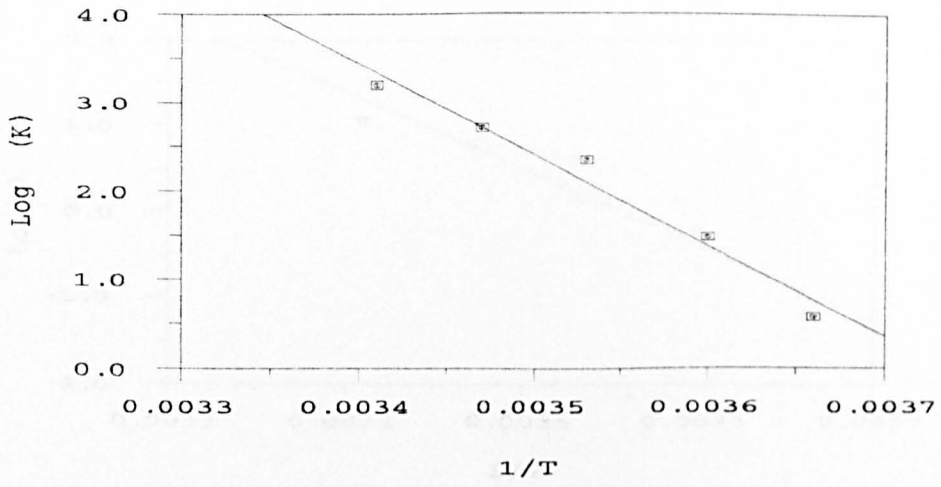



Fig 4.16 Arrhenius plot for nitrate formation in Darvel soil

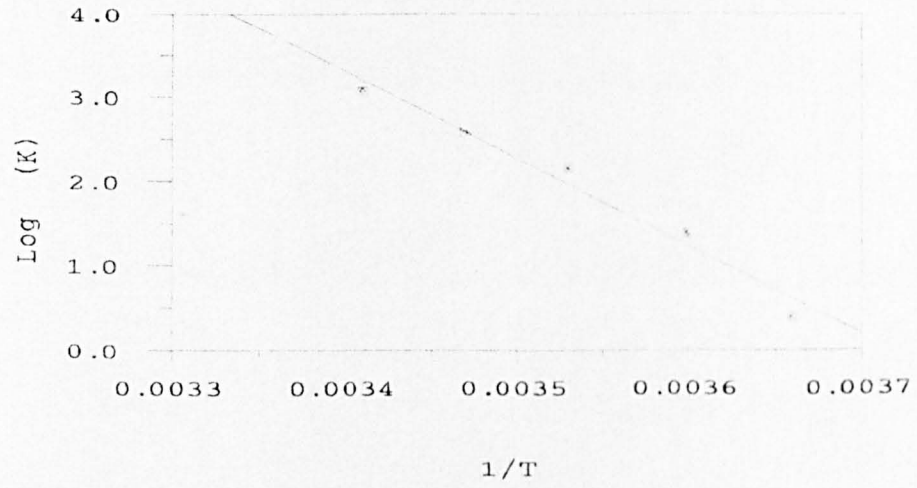



Fig 4.17 Arrhenius plot for ammonium disappearance in Darvel soil

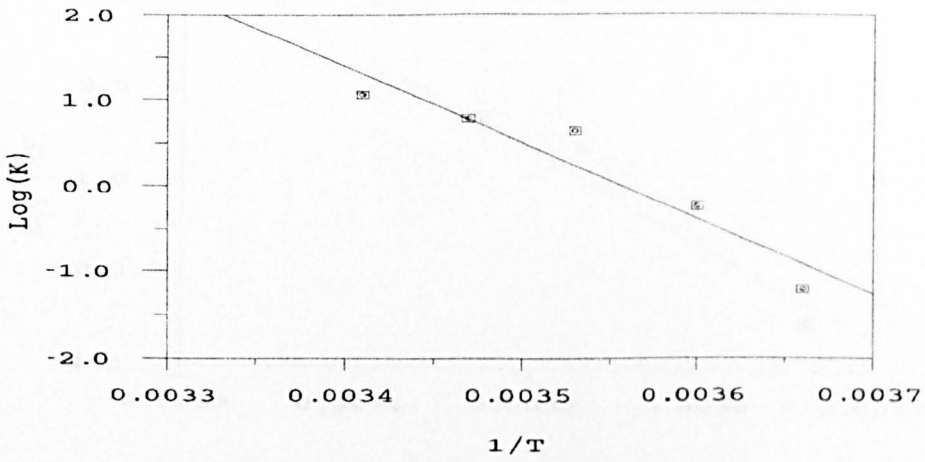



Fig 4.18 Arrhenius plot for mineralisation in Darvel soil

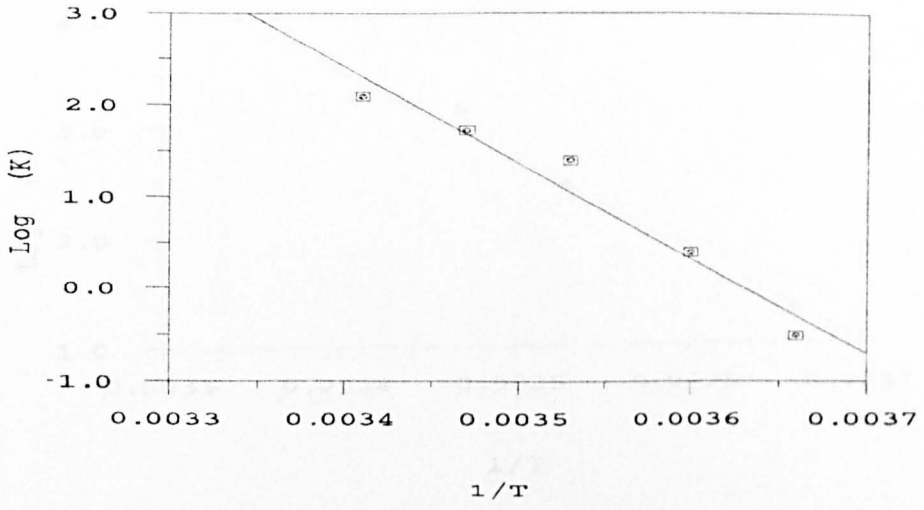



Fig 4.19 Arrhenius plot for nitrate formation in Freckenham soil

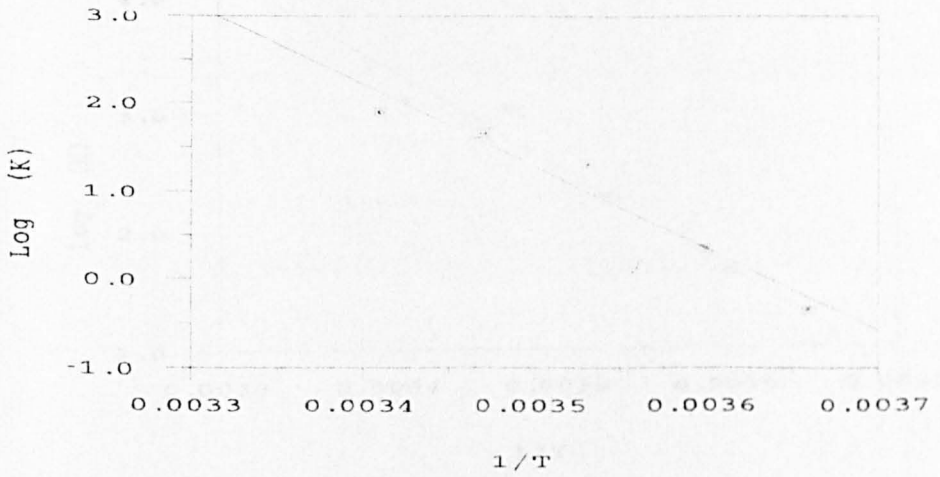



Fig 4.20 Arrhenius plot for ammonium disappearance in Freckenham soil

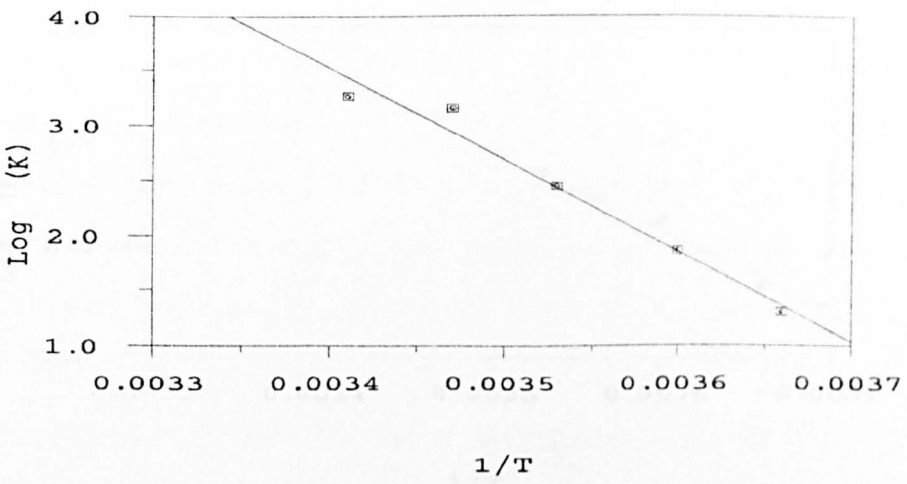



Fig 4.21 Arrhenius plot for nitrate formation in Darlieth soil

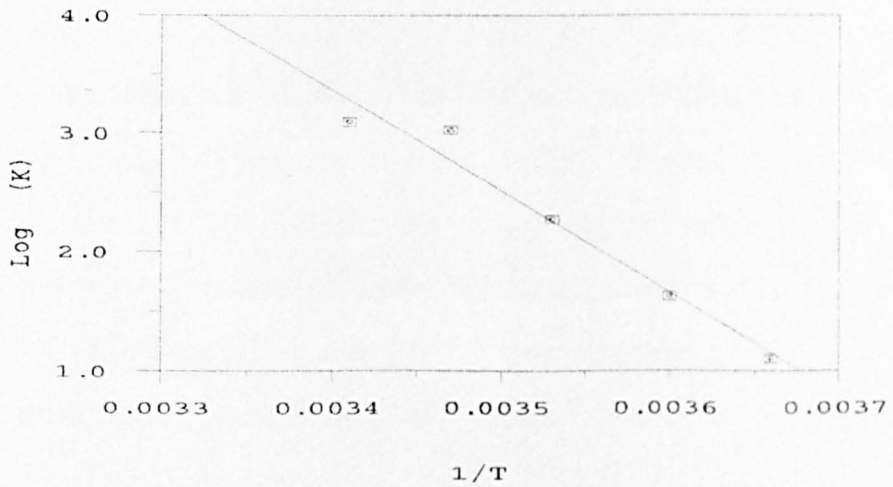



Fig 4.22 Arrhenius plot for ammonium disappearnace in Darlieth soil

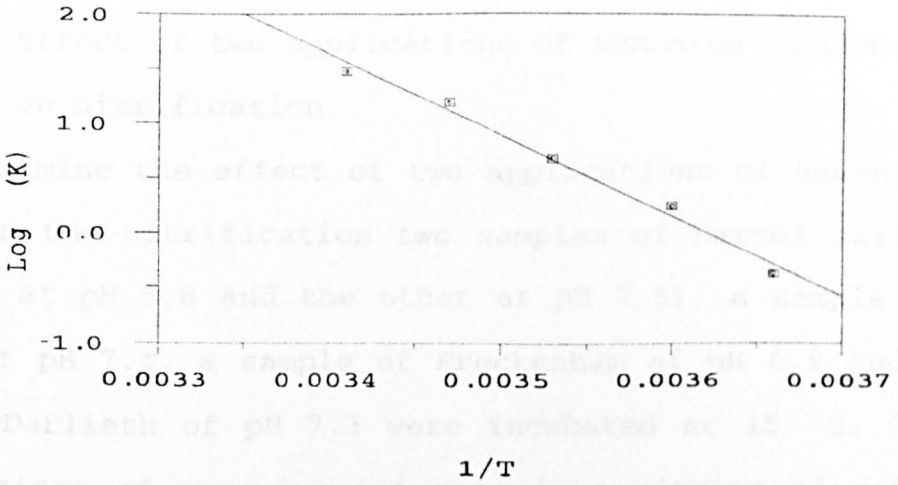



Fig 4.23 Arrhenius plot for mineralisation in Darlieth soil

### 4.3.3. Effect of two applications of ammonium sulphate on nitrification.

To examine the effect of two applications of ammonium sulphate on the nitrification two samples of Darvel series soils (one at pH 5.8 and the other at pH 7.5), a sample of Midelney of pH 7.4, a sample of Freckenham of pH 6.9 and a sample of Darlieth of pH 7.3 were incubated at 15 °C. The transformations of ammonium nitrogen into nitrate nitrogen and the effect of pH with passage of time is shown in Figures 4.24 to 4.28. The figures were made on the same scale for all soils for easy comparison. Figure 4.24 shows the first and second applications of ammonium sulphate in Darvel soil of pH 5.8. Here each application took approximately 10 days for complete conversion from ammonium to nitrate. In the Darvel soil at pH 7.5 the period was reduced from 10 days to 6 days as shown in Figure 4.25. A wide range of nitrification rates were observed in the experiment. Oxidation of 100 mg $kg^{-1}$ of ammonium took up to 20 days in Freckenham and 4 days for Midelney soil series. Figures 4.25, 4.27 and 4.28 for the Darvel (pH 7.5), Freckenham and Darlieth series soils show significant increases in total inorganic nitrogen following the second application due to very high measured nitrate values. Consequently it is thought that this was due to an analytical error in the measurement of nitrate. Therefore nitrate formation rates were not calculated for the second application for these soils. This does not affect the calculation of rates of ammonium disappearance.

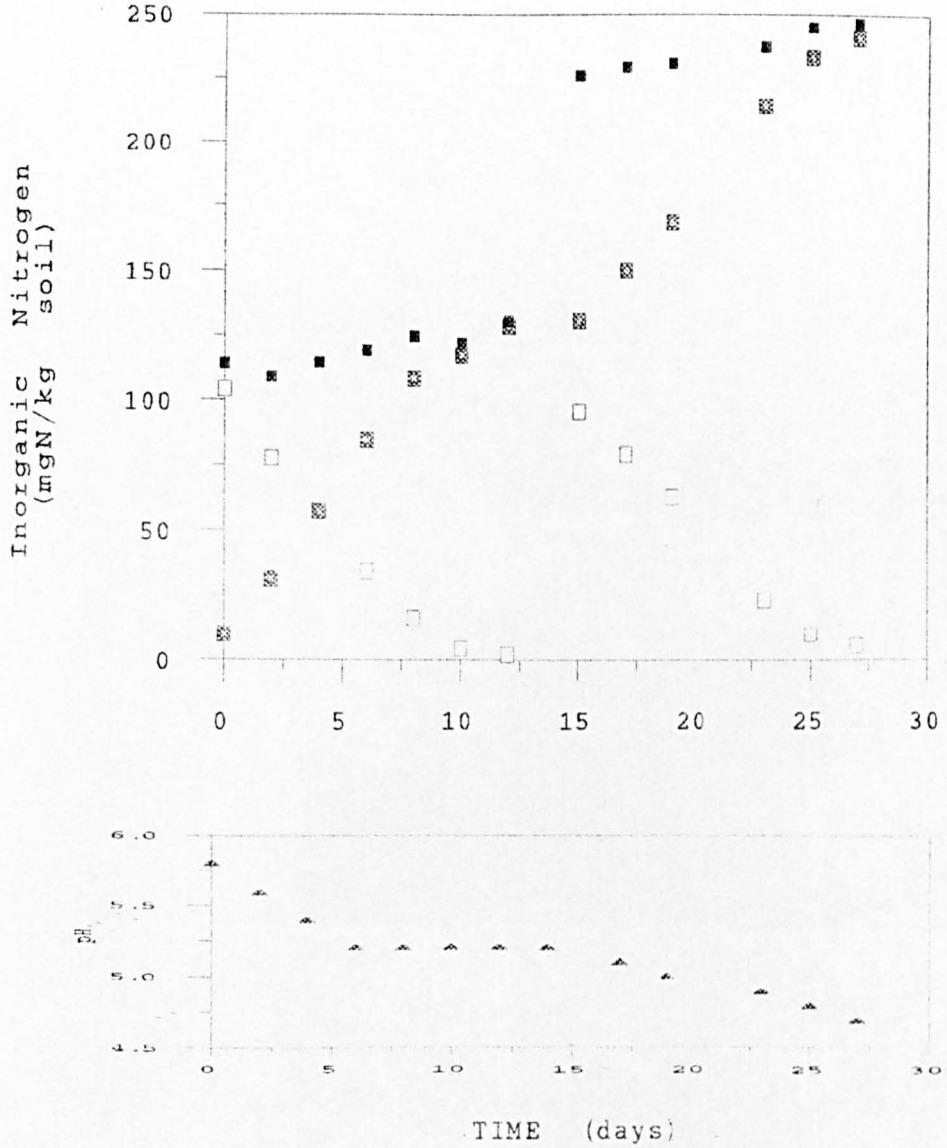



Fig 4.24 Nitrification in Darvel soil (pH 5.8) with repeated application of ammonium sulphate

□ Ammonium-N
▨ Nitrate-N
■ Total inorganic-N

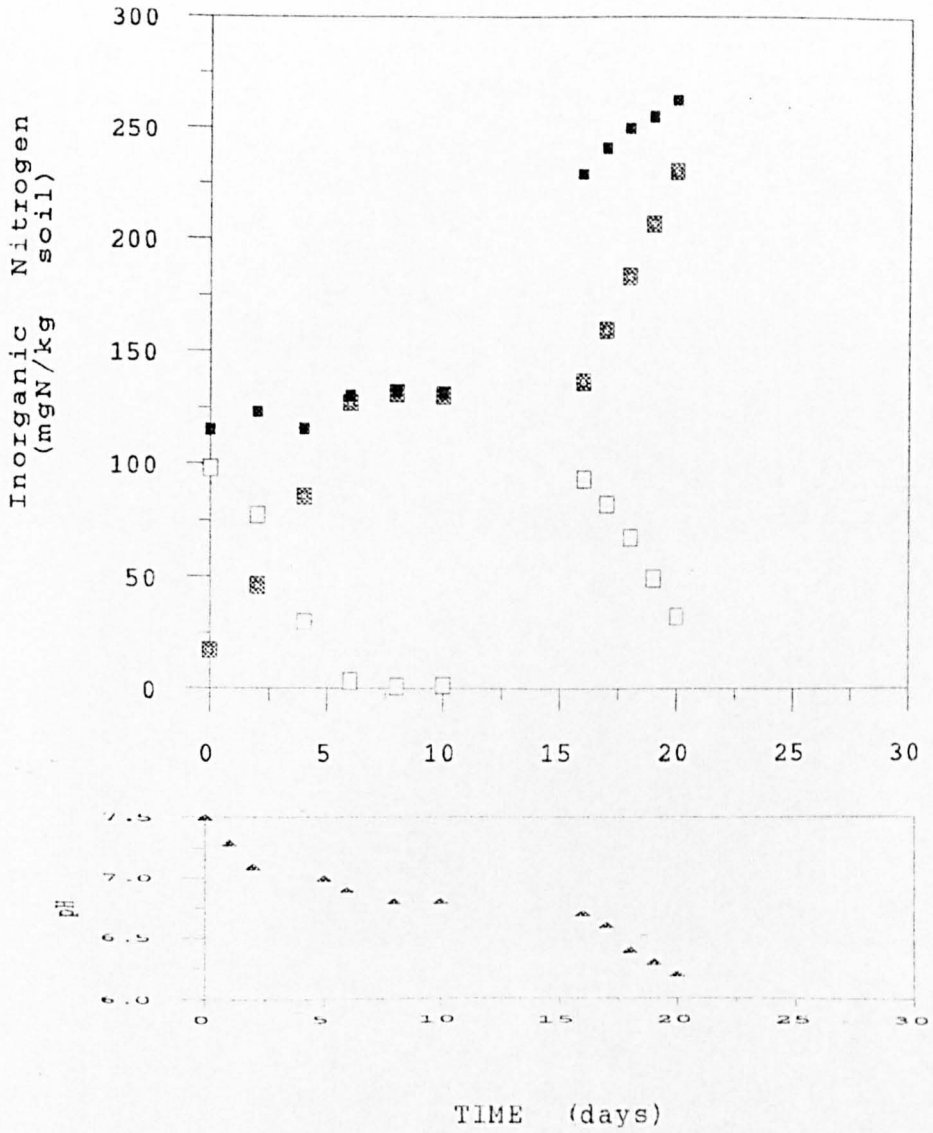



Fig. 4.25 Nitrification in Darvel soil (pH 7.5) with repeated appliction of ammonium sulphate

Ammonium-N
Nitrate-N
Total inorganic-N

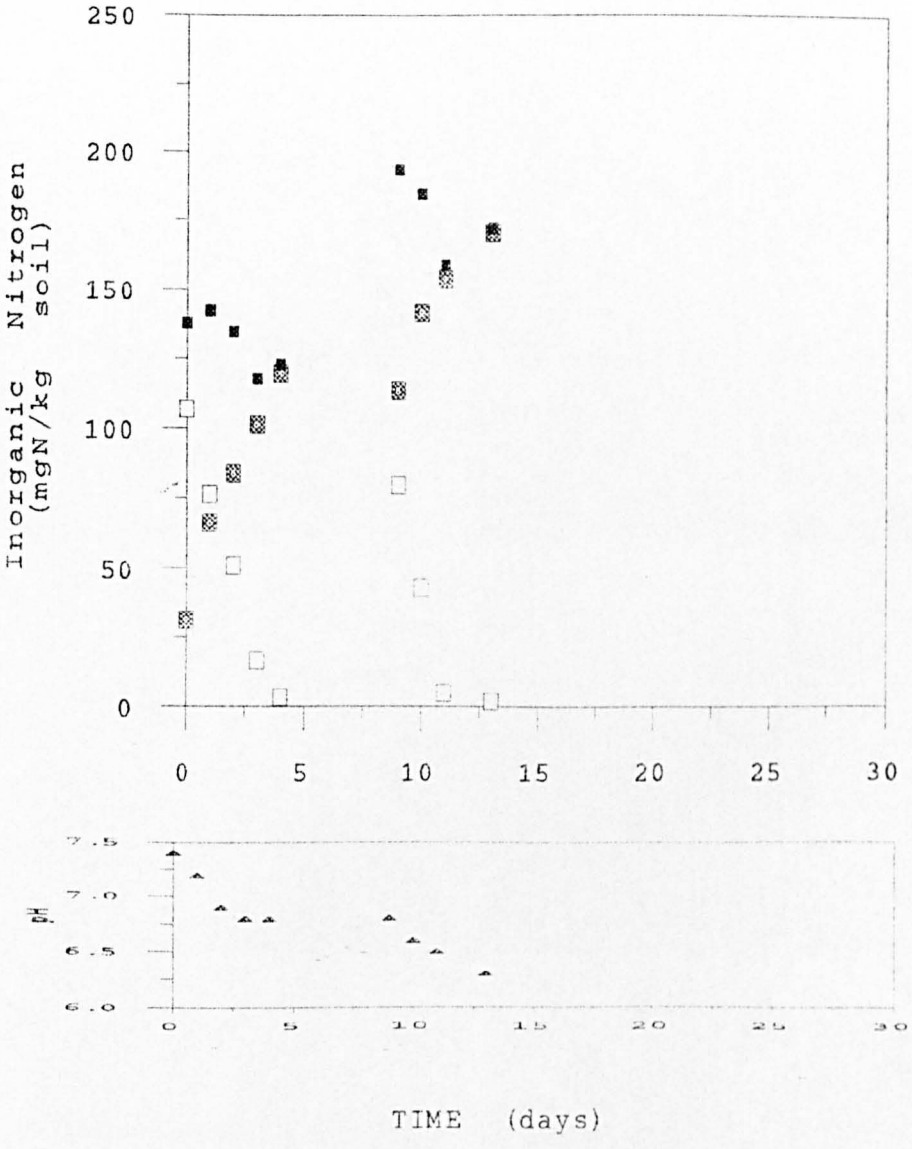



Fig. 4.26 Nitrification in Midelney soil with repeated application of ammonium sulphate

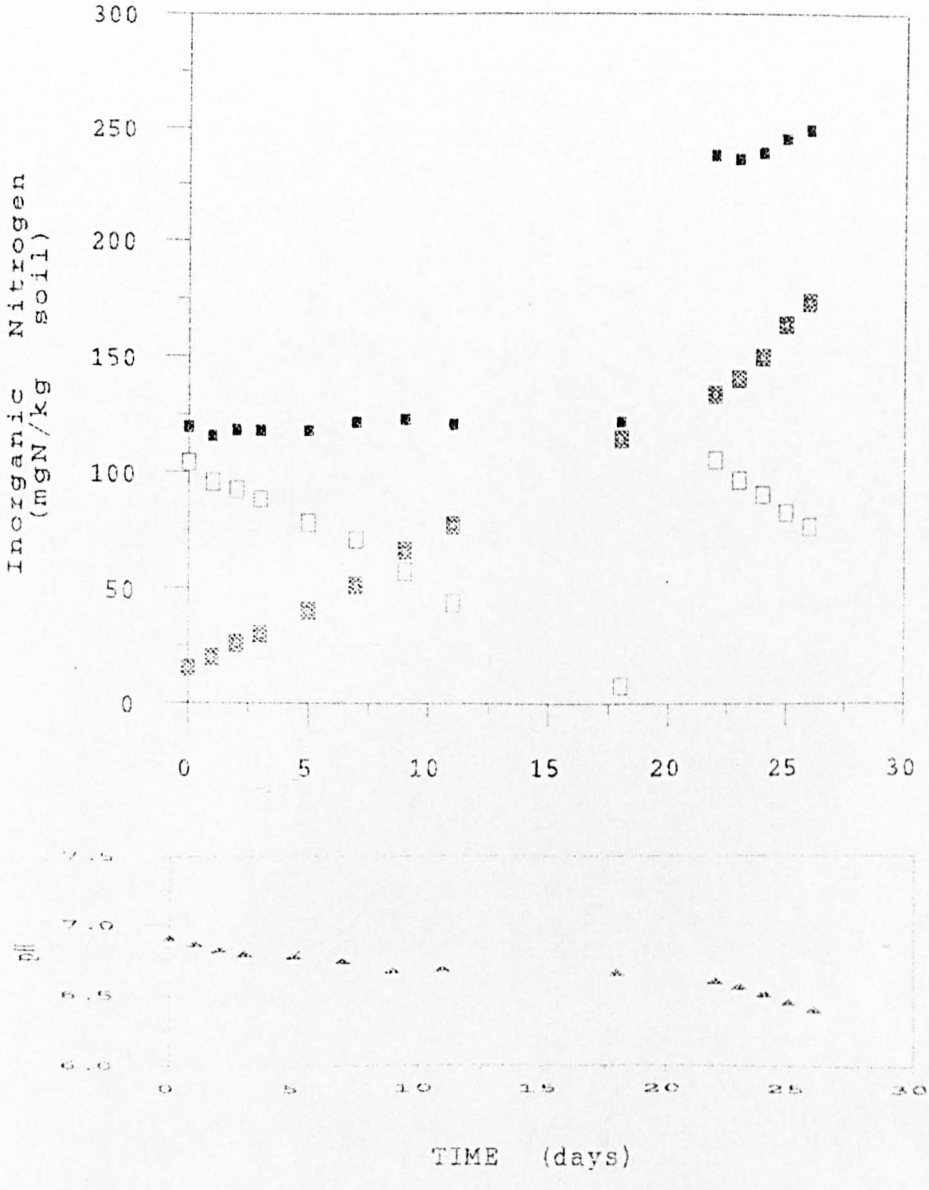



Fig. 4.27 Nitrification in Freckenham soil with repeated appliction of ammonium sulphate

□ Ammonium-N
▨ Nitrate-N
▪ Total inorganic-N

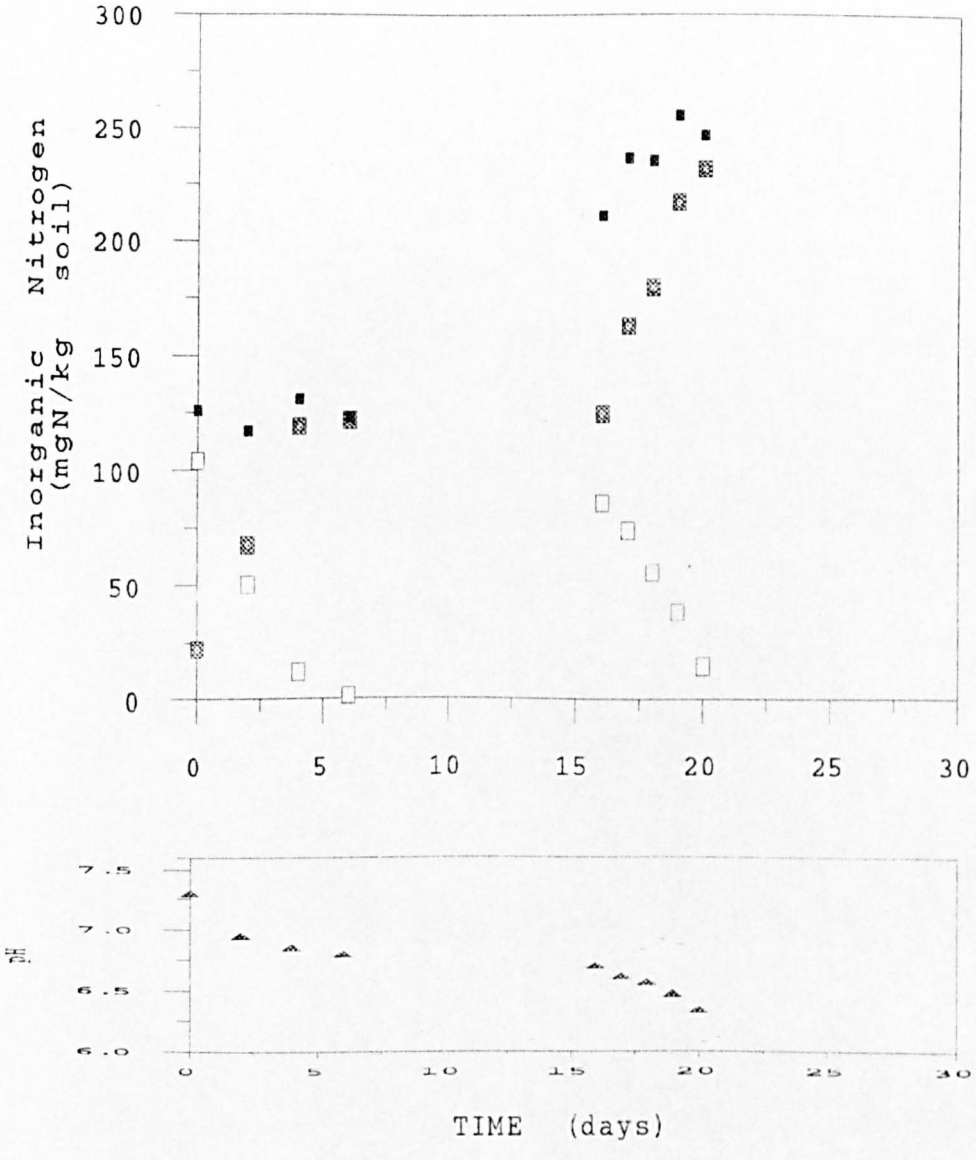



Fig. 4.28 Nitrification in Darlieth soil with repeated application of ammonium sulphate

The ammonium disappearance and nitrate formation rates were calculated for the first application for all soils but only ammonium disappearance for the second application for the Darvel ( pH 7.5 ) Freckenham and Darlieth soil series. Table 4.8 shows the means of the zero order rate constants.

Table 4.8 Effect of two applications of ammonium sulphate on nitrification

| | Zero order rates constant ( mg N $kg^{-1}$ soil $day^{-1}$) | |
|---|---|---|
| | First application | Second application |
| Darvel (pH 5.8) | | |
| Nitrate formation rate | 12.5 b | 10.3 a |
| Ammonium disappearance rate | 10.6 b | 8.8 a |
| Darvel (pH 7.5) | | |
| Nitrate formation rate | 17.6 | - |
| Ammonium disappearance rate | 16.3 a | 14.9 a |
| Midelney | | |
| Nitrate formation rate | 27.1 a | 30.1 a |
| Ammonium disappearance rate | 27.2 a | 28.5 a |
| Freckenham | | |
| Nitrate formation rate | 5.6 | - |
| Ammonium disappearance rate | 5.2 a | 5.9 a |
| Darlieth | | |
| Nitrate formation rate | 23.6 | - |
| Ammonium disappearance rate | 20.3 a | 17.9 a |

1) Data in a row with same subscript letter are not significantly different at 5% level (pooled t-test).

The t test on the rates of ammonium disappearance and nitrate formation showed that the effect of two applications of ammonium was nonsignificant for Darvel (pH 7.5), Midelney, Freckenham and Darlieth soil samples. In Darvel (pH 5.8) soil a significant inhibitory effect on the second application was found on both ammonium disappearance and nitrate formation. The reason for the inhibition of nitrification in the second application in the Darvel soil (pH 5.8) was due to a fall in pH. In the Darvel soil (pH 5.8 ) the pH decreased from 5.8 to 5.2 in the first incubation. The second application was applied at pH. 5.2 and pH fell further to pH 4.8. In acid environments, nitrification proceeds slowly even in the presence of adequate supply of substrate, and the responsible species are rare or totally absent at great acidities. Frederick et al. (1956) reported that nitrification rate decreases with decrease in soil pH. Flowers and O'Challaghan (1983) reported that nitrification rate constants relate to initial pH although, as there was no change in nitrification during incubation, the rates were not affected by the decrease in pH resulting from nitrification. They suggested that there was a small increase in the population of nitrifiers and that growth would be balanced by the effect of the fall in pH. Cooper (1975) attributed the differences in nitrification of ammonium added as ammonium sulphate or pig slurry to the rise in soil pH caused by slurry addition. This was partly an inhibitory effect of high pH caused by slurry addition to an initially neutral soil but the increased

nitrification in a slurry-treated acid soil was attributed directly to the effect of increased soil pH on nitrification.

The reason for measuring nonsignificant differences in the rates of nitrification between first and second application of ammonium sulphate in other soils was probably the balance between inhibition of nitrifying bacteria due to fall in pH and growth of the organisms in the presence of adequate availability of ammonium.

## 4.4 CONCLUSIONS

From the present study the following conclusions are made:-

1. Fresh soil samples nitrified applied ammonium sulphate more quickly compared to air dried soil samples. The air dried soil samples showed a lag period in nitrification activity and nitrate formation appeared to fallow a sigmoid curve whereas in fresh soil samples no lag period was observed and nitrate formation was linear up to complete conversion of applied ammonium sulphate into nitrate nitrogen. The fresh soil samples showed a better reproducibility of nitrification rates in all soils and the data fitted better to a straight line. More points of the data from fresh soils samples fitted a straight line for calculating zero order rate constants compared to air dried samples. The nitrification rates were higher in the fresh subsamples compared to the air dried soil subsamples. However air dried soil samples had a higher mineralisation rate than the fresh soil samples.

2. The nitrification and mineralisation rates increased as the temperature increased in all soils tested. The nitrification rates at 0 °C in Darvel, Freckenham and Darlieth soil samples indicated that the nitrifying bacteria can survive and can nitrify applied ammonium sulphate down to the lowest temperature tested. The rates of ammonium disappearance and nitrate formation gave a good fit to the Arrhenius relationship for all soils over all the temperatures tested ( 0, 5, 10, 15, 20 °C ).

3. In soils treated with two applications of ammonium sulphate the effect of the second application was influenced by the initial soil pH:-

In soils having an initial pH near to pH 7 the nitrification rates were not significantly different between the first and second application however, for the Darvel soil (initial pH 5.8) a significant inhibitory effect was observed on nitrification of the second application. This was attributed to the fall in pH during the first period of incubation.

# CHAPTER 5

## AMMONIUM CONTAMINATED LAND AT A FORMER NYLON FACTORY SITE

### 5.1 INTRODUCTION

In summer 1993 the Clyde River Purification Board conducted a survey of an area close to ICI Explosives, Nobel's Explosives Company's Ardeer site, at Stevenson, Ayrshire, with respect to making the area a nature conservation area. At the northern edge of the site is a railway line and just across the railway line is the Master Gott river. They found elevated levels of ammonia, iron and sulphide in the Master Gott river adjacent to the former Nylon plant on the ICI site. Iron and sulphide were expected due to the extensive mine workings in the area, but high levels of ammonia were unexpected. The board approached ICI Explosives in order to trace the source of the elevated ammonia level in the Master Gott. The ICI Explosives Company conducted a preliminary analysis of ground water samples as shown in Table 5.1. The locations of the sampling points for the water samples collected are shown in Map 5.1.

Table 5.1 Analysis of ground water samples collected from the former nylon site at Ardeer during November and December 1993.

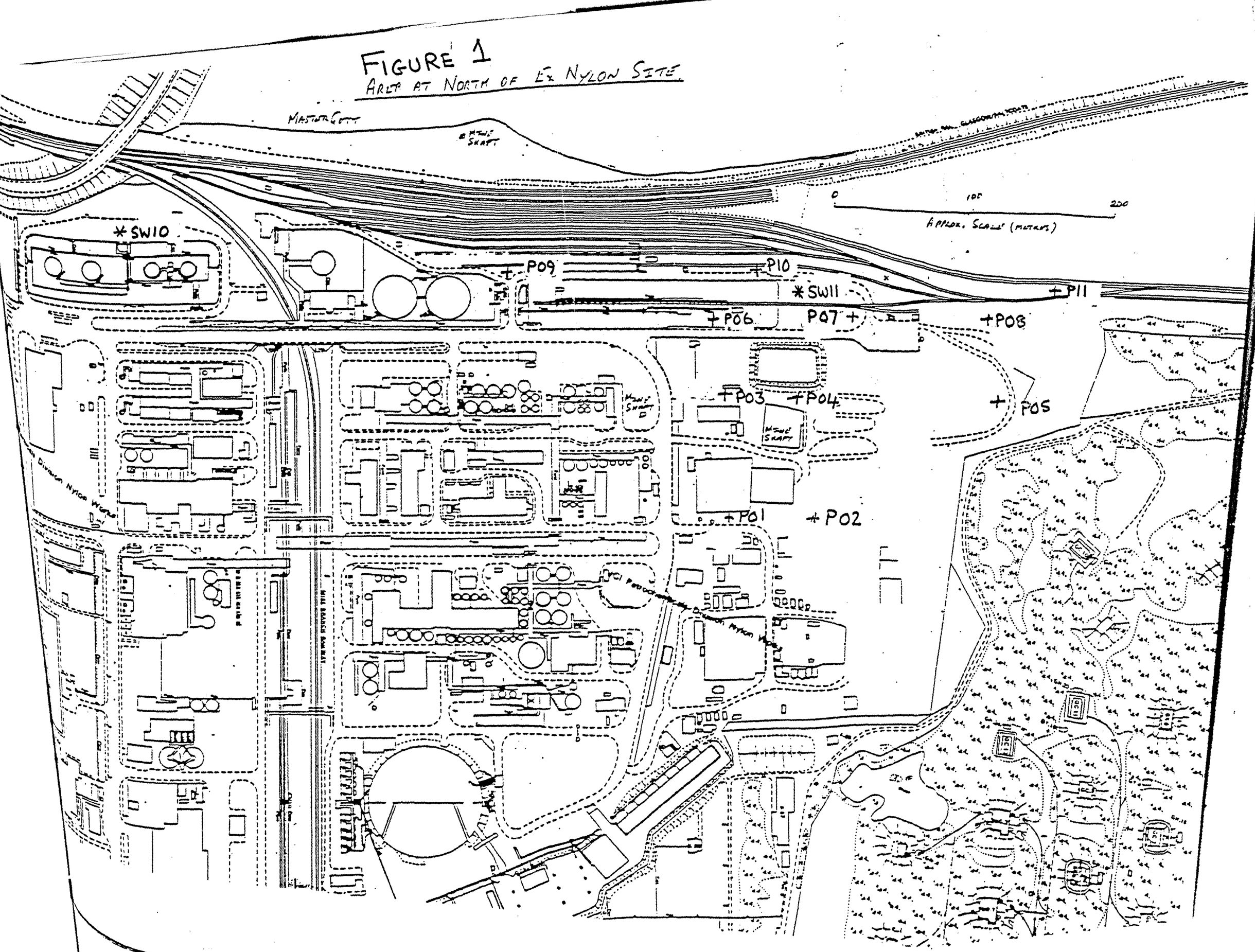
| Date | Sample point | Ammonium (mg $l^{-1}$) | pH |
|---|---|---|---|
| 2.11.93 | SW10 | 10 | 7.5 |
| | SW11 | 40 | 8.0 |
| 30.11.93 | SW10 | 10 | - |
| | SW11 | 36 | - |
| 4.12.93 | PO1 | <1 | 6.7 |
| | PO2 | <1 | 6.7 |
| | PO3 | 5 | 6.9 |
| | PO4 | 2 | 6.9 |
| | PO5 | <1 | 8.5 |
| | PO6 | 12 | 7.7 |
| | PO7 | 3 | 7.3 |
| | PO8 | <1 | 7.3 |
| | PO9 | 90 | 7.7 |
| 5.12.93 | P10 | 8 | 6.9 |
| | P11 | 4 | 7.4 |

These ammonium levels (upto 90 mg $l^{-1}$) are unusually high, as the ammonium would normally be expected to be held on cation exchange sites. The main possible sources of ammonia contamination in ground water are usually land-based inputs, chiefly sewage and run-off from farmland. The latter will include ammonia from livestock wastes, which contain appreciable amounts of ammonia, from spreading of sewage sludge, and to some extent from the proportion of fertilisers which is not utilised by the crop. Another source of ammonia is the by-products from industrial processes such as coke production and the fertiliser and textile industries.

Map 5.1 Sampling points of preliminary water samples.

# FIGURE 1

AREA AT NORTH OF EX NYLON SITE



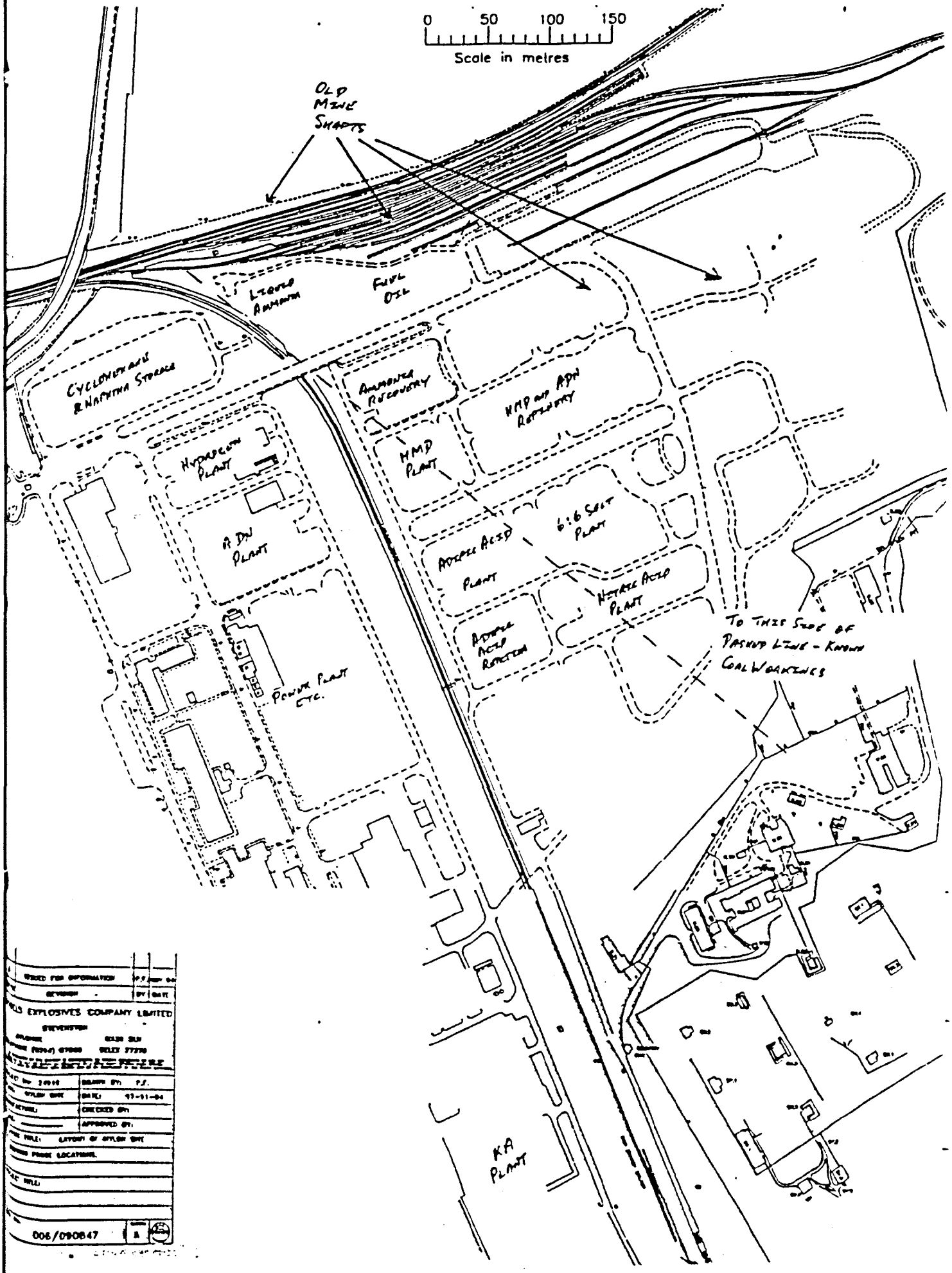
Before industrial use, up to the early years of this century, the site was farmed and, in the northern section, mined for coal. The area north of the site was extensively worked and a number of small canals were dug for moving the coal, one of which remains and can still be seen as the Master Gott river. The ICI site was extended with the development of a plant for producing nylon. In 1964 the nylon factory construction work was started and it was completed in 1968. Ammonia was brought in by rail for use in the nylon production plant and an associated nitric acid plant. Nylon was produced for 14 years (1968-1981). The nylon plant was demolished in 1981 leaving the nitric acid plant in operation. The ammonia storage was closed in about 1984 and the nitric acid plant was closed in 1992. Demolition of these facilities was carried out in 1993. The ammonium storage area and other facilities are shown on the Map 5.2.

A study was planned to investigate the possible sources, distribution and concentration of ammonium in soil and ground water in the former nylon factory site. This work was fitted into a wider programme of site investigation carried out by ICI to investigate other possible contamination on the former nylon site.

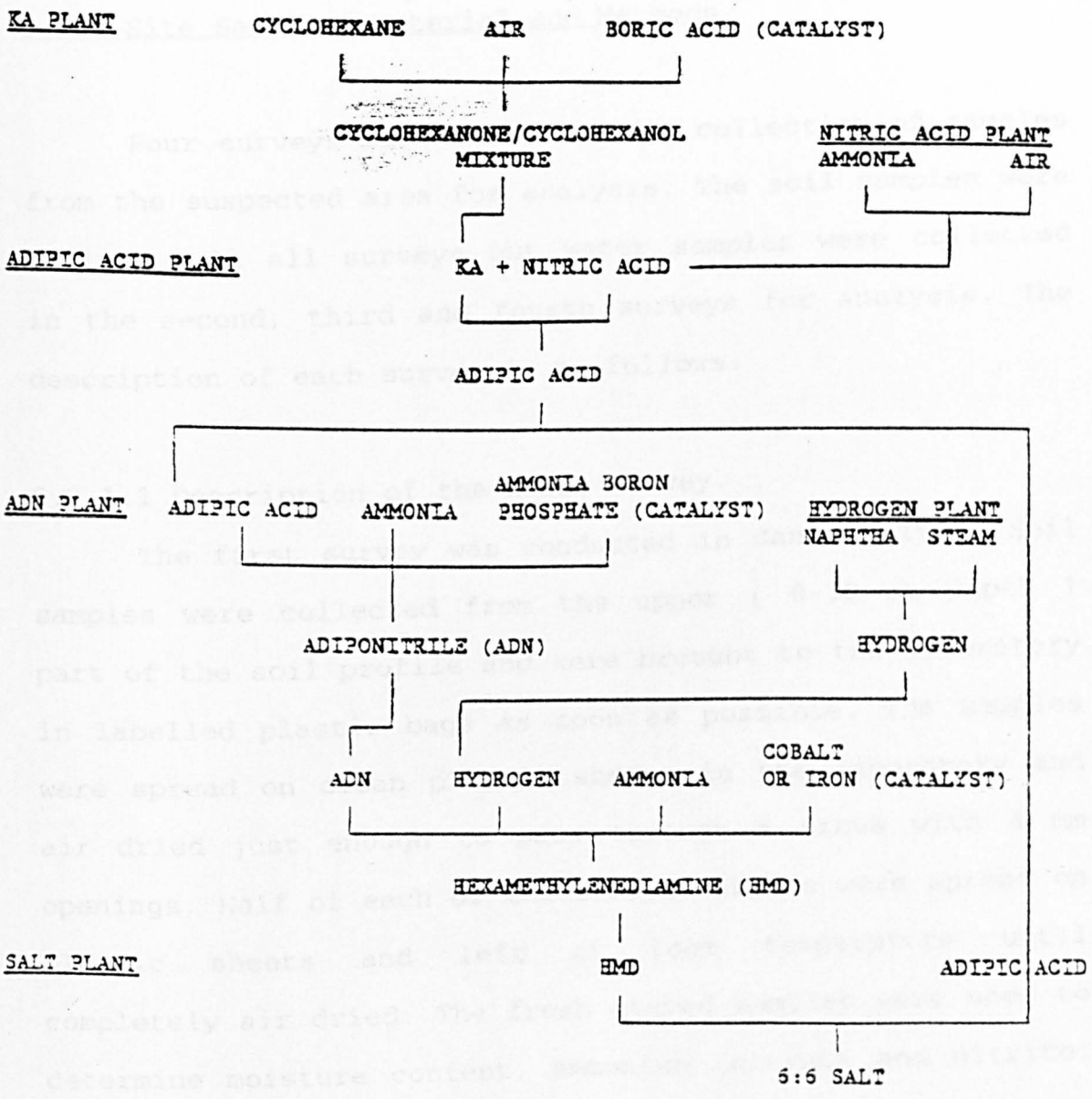
The investigation was made in four stages. In the first investigation the ammonium levels in the surface soil were measured, to determine spillage of ammonia on the nylon site. The second survey was conducted by collecting samples near the previous ammonia storage tanks from the subsoil down to the ground water to find ammonium levels in

the different horizons. Due to the difficulty in sampling by trial pits a third comprehensive investigation was made using geoprobe cores to find ammonium levels in the suspected area. A fourth survey was conducted to find out ammonium level at greater depths and to fill in some gaps in the third survey.

Map 5.2 Nylon Factory site.


0 50 100 150
Scale in metres
OLD MINE SHAFTS
LIQUID AMMONIA
FUEL OIL
CYCLOHEXANE & NAPHTHA STORAGE
AMMONIA RECOVERY
HMD AND ADN REFINERY
HMD PLANT
HYDROGEN PLANT
ADN PLANT
ADIPIC ACID PLANT
6:6 SALT PLANT
NITRIC ACID PLANT
ADIPIC ACID REFINERY
POWER PLANT ETC.
TO THIS SIDE OF PASHLEY LANE - KNOWN COAL WORKINGS
KA PLANT
ISSUED FOR INFORMATION
REVISION
ELS EXPLOSIVES COMPANY LIMITED
STEVENSTON
DRAWN BY: P.F.
DATE: 93-11-04
CHECKED BY:
APPROVED BY:
LAYOUT OF OFFLOW SITE
006/090847
A

## NYLON 6:6 SALT MANUFACTURING PROCESS OUTLINE




On one of the attached drawings I have indicated where the different plants were situated. In the outline above I have omitted the various refining and purification stages.

## 5.2 METHODS AND MATERIALS

### 5.2.1 Site Sampling Material and Methods.

Four surveys were conducted for collection of samples from the suspected area for analysis. The soil samples were collected in all surveys but water samples were collected in the second, third and fourth surveys for analysis. The description of each survey is as follows:

#### 5.2.1.1 Description of the first survey.

The first survey was conducted in January 1994. Soil samples were collected from the upper ( 0-30 cm depth ) part 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 in the laboratory and air dried just enough to pass through a sieve with 4 mm openings. Half of each of the sieved samples were spread on plastic sheets and left at room temperature until completely air dried. The fresh sieved samples were used to determine moisture content, ammonium, nitrate and nitrite. The air dried samples were used to determined pH, conductivity and for storage. The sample identifications are supplied in Table 5.2 and location of sampling points in Map 5.3.

Map 5.3 Sampling points of first survey.

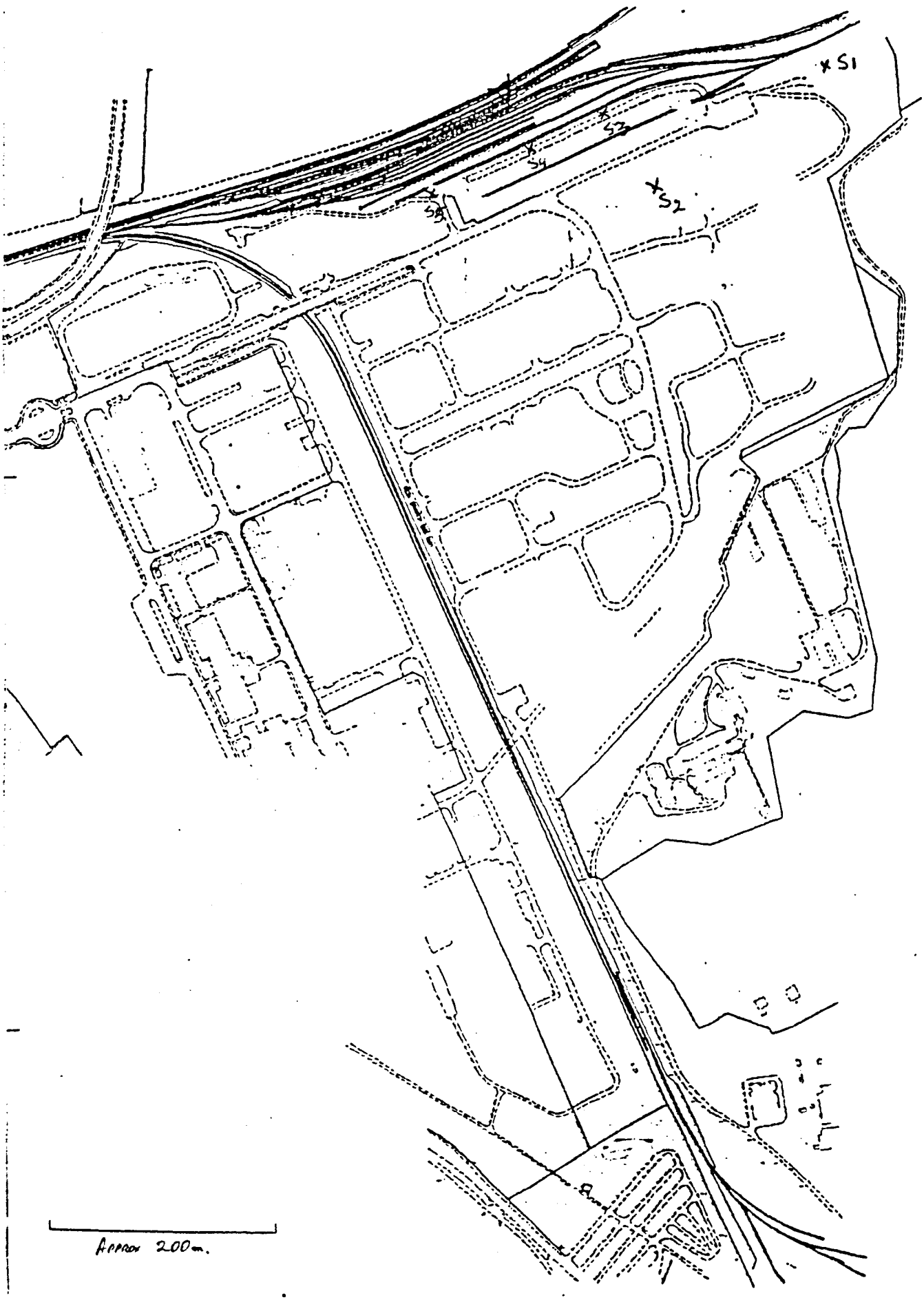

S1
S2
S3
S4
S5
Approx 200m.

Table 5.2 Sample identification for the first survey.

| No | Location | Sample ID | Depth (cm) |
|---|---|---|---|
| 1 | S1 | 1A | 0-15 |
| 2 | | 1B | 40-50 |
| 3 | S2 | 2A | 0-15 |
| 4 | | 2B | 35-45 |
| 5 | S3 | 3A | 0-12 |
| 6 | | 3B | 15-25 |
| 7 | S4 | 4A | 0-15 |
| 8 | | 4B | 15-30 |
| 9 | S5 | 5A | 0-10 |
| 10 | | 5B | 15-25 |

5.2.1.2 Description of the second survey.

The second survey was conducted in April 1994 by digging three trial pits to the depth of the ground water table using a JCB digger. Soil samples for the different horizons were collected in labelled plastic bags and free ground water samples in 250 ml labelled plastic bottles. All the samples were brought to the laboratory as soon as possible. The soil samples were spread on clean plastic sheets in the laboratory. The pore water was extracted from the very wet soil samples taken from below the water table. The pore water samples from the very wet soil samples were extracted using a Sartorius polycarbonate vacuum filtration system using Whatman glass fibre filter paper GF/C into polystyrene 30 ml straight sided universal vials.

The glass fibre filter paper was placed in the filtration unit. The filtration unit and filter paper were washed first with deionized water then approximately 100 ml pore water was extracted from the soil. This first porewater sample was discarded then a second porewater sample was extracted from the soil and retained for analysis.

All the soil samples were air dried just enough to pass through a sieve with 4 mm openings. The samples were then stored at 2°C if not analysed at once. Half of each of the sieved soil samples were spread on plastic sheets and left at room temperature until completely air dried. The fresh soil samples were used for the determination of moisture content, loss of ignition, ammonium, nitrate and nitrite. The air dried soil samples were used for the determination of pH, conductivity, cation exchange capacity and for mechanical analysis. Details of the soil and water samples collected are in Tables 5.3 and 5.4 respectively. The location of the three trial pits is shown in Map 5.4.

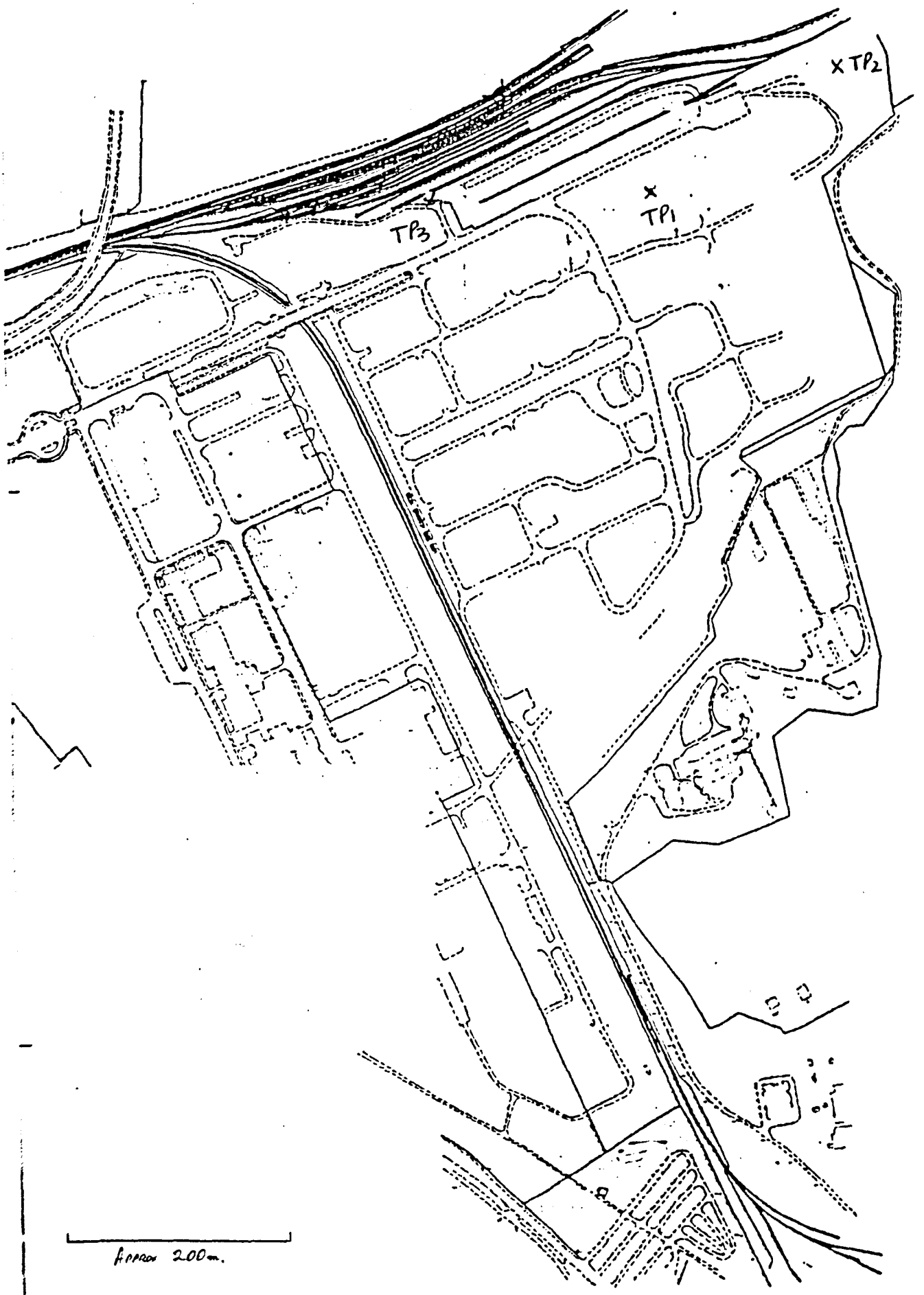
Table 5.3 Soil sample identification from the second survey.

| Location | Sample ID | Depth (cm) | Description |
|---|---|---|---|
| TP1 | TP1A | 35-90 | Yellow sand |
| | TP1B | 91-155 | Black, greasy ash and sand mixture |
| | TP1C | 156-300 | Grey brown wet sand |
| | TP1D | >301 | Saturated sand |
| TP2 | TP2A | 35-70 | Black soil sand |
| | TP2B | 71-100 | Yellow sand |
| | TP2C | 101-160 | Grey sand |
| | TP2D | 161-180 | Orange sand |
| | TP2E | 221-240 | Greyer wet sand |
| | TP2F | >350 | Saturated sand |
| TP3 | TP3A | 35-80 | Mixed soil sand |
| | TP3B | 81-120 | Stone sand |
| | TP3C | 121-140 | Grey sand |
| | TP3D | 141-160 | Oily sheen sand |

Table 5.4 Water sample identification from the second survey.

| Location | Sample ID | Depth (cm) | Description |
|---|---|---|---|
| TP1 | TP1CW | 156-300 | Extractable pore water from soil sample TP1C |
| | TP1DW | >301 | Extractable pore water from soil sample TP1D |
| | TP1GW | >301 | Ground water |
| TP2 | TP2FW | >350 | Extractable pore water from TP2F |
| | TP2GW | >350 | Ground water |
| TP3 | PT3CW | 121-140 | Extractable pore water from soil sample TP3C |
| | TP3DW | 141-160 | Extractable pore water from soil sample TP3D |
| | TP3GW | 160 | Ground water |

Map 5.4 Sampling points of second survey.


TP2
TP1
TP3
Approx 200m.

5.2.1.3 Description of soil samples from the third survey.

The third survey was conducted in June 1994 by taking sixty one Geoprobe cores at different sites as shown in Map 5.5. Samples at 0.5 m spacing down to below the ground water table were collected and brought to the Agricultural, Food and Environmental Chemistry Laboratory, Glasgow Univesity in labelled plastic tubes in an insulated cooler as soon as possible. The ground water samples were collected and analysed by the Environmental Chemistry Laboratory of ICI at Ardeer. The soil samples were stored in a deep freezer. Before taking samples for extraction for measuring inorganic nitrogen the soil sample tubes were taken out of the deep freezer and left at laboratory temperature for 2 hours to thaw and then mixed. 2.5 g of soil was taken from each tube for extraction. The remaining soil samples were divided into three portions. One was for the measurement of moisture content and organic matter ( loss of ignition ). The second portion was spread on clean plastic sheets at laboratory temperature until completely air dried for the measurement of pH and conductivity. The third portion of each sample was left in the freezer. Sample identification, probe hole number and a brief description of the samples is given in Table 5.5.

Map 5.5 Sampling points of third survey.

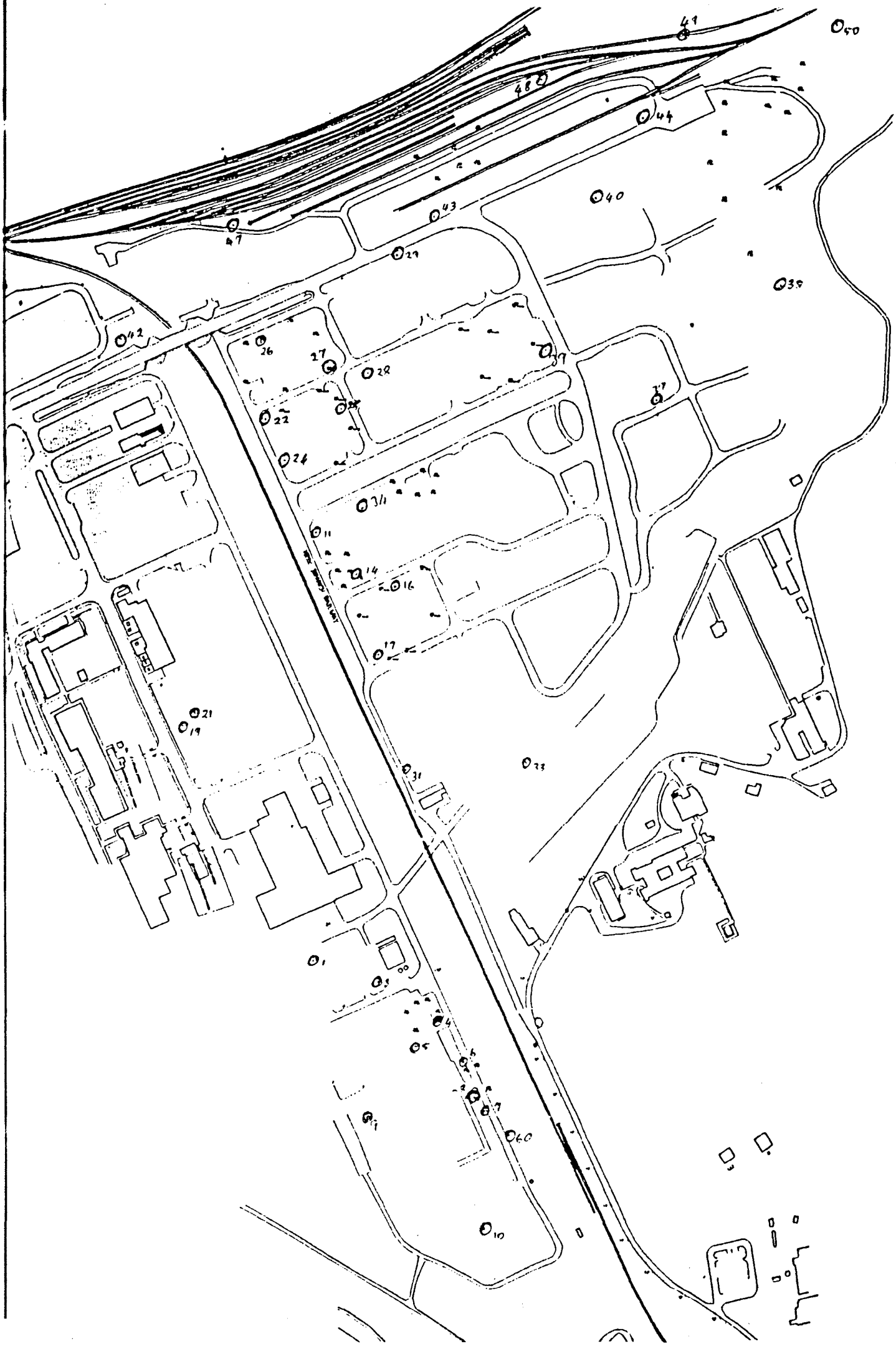

41
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Table 5.5 Description of soil sample for the third survey.

| Probe No. & Sample ID | Depth (m) | Description |
|---|---|---|
| 1A | 1.0-1.5 | Yellow brown sand |
| 1B | 2.7-3.2 | Brown sand with small stones |
| 3A | 1.0-1.3 | Brown clear sand |
| 3B | 2.7-3.0 | Brown sand mixed with coal |
| 4A | 1.0-1.3 | Black grey sand |
| 4B | 2.7-3.0 | Black brown sand |
| 5A | 1.0-1.5 | Dark brown sand |
| 5B | 2.5-3.0 | Brown sand and low organic matter |
| 6A | 1.0-1.5 | Dark sand with black coal and stones |
| 6B | 2.0-2.5 | Brown sand |
| 6C | 3.0-3.5 | White sand |
| 6D | 4.0-4.5 | White coarse sand |
| 6E | 5.0-5.5 | Grey sand |
| 7A | 1.0-1.3 | Brown sand |
| 7B | 2.7-3.0 | Brown sand |
| 8A | 1.0-1.3 | Dark brown sand |
| 8B | 2.7-3.0 | Brown sand |
| 9A | 1.0-1.5 | Coarse white sand |
| 9B | 2.7-3.2 | Green sand |
| 10A | 1.0-1.5 | White sand |
| 10B | - | Dark brown sand |
| 11A | 1.0-1.3 | Brown sand mixed with a little coal |
| 11B | 2.7-3.2 | Brown sand mixed with coal |
| 14A | 1.0-1.3 | Yellow sand |
| 14B | 2.7-3.0 | Yellow sand |
| 16A | 1.5-1.9 | Grey sand with stones |
| 16B | 2.7-3.1 | Grey sand and organic material |
| 17A | 1.0-1.3 | Grey sand |
| 17B | 2.7-3.0 | Dark grey greasy sand |
| 19A | 1.0-1.3 | Top soil |
| 19B | 2.7-3.0 | White sand |
| 21A | 1.0-1.3 | Brown dark sand |
| 21B | 2.7-3.0 | Grey sand |

| Probe No. & Sample ID | Depth (m) | Description |
|---|---|---|
| 22A | 1.0-1.3 | Yellow sand |
| 22B | 2.7-3.0 | Yellow sand |
| 25A | 1.0-1.3 | Brown sand |
| 25B | 2.7-3.0 | Fine sand mixed with coal |
| 26A | 1.0-1.3 | Coal and black sandstones |
| 26B | 2.7-3.0 | White sand |
| 27A | 1.0-1.3 | Brown sand |
| 27B | 2.7-3.0 | Brown sand |
| 28A | 1.6-2.2 | Yellow red sand |
| 28B | 2.7-3.0 | Brown sand mixed with coal |
| 29A | 1.0-1.5 | Blue grey rock |
| 29B | 2.7-3.0 | White sand |
| 29C | 3.0-3.5 | Brown white sand |
| 29D | 4.0-4.5 | Black brown sand |
| 29E | 5.0-5.5 | Black brown mixed sand |
| 31A | 1.0-1.7 | Dark yellow sand |
| 31B | 2.7-3.0 | Yellow sand |
| 33A | 1.0-1.3 | Coal and dark grey sand |
| 33B | 2.0-2.3 | Dark grey sand |
| 33C | 2.7-3.0 | Grey sand |
| 33D | 3.0-3.3 | White sand |
| 33E | 4.0-4.3 | White sand |
| 33F | 5.0-5.3 | White sand |
| 34A | 1.0-1.3 | Fine yellow sand |
| 34B | 2.7-3.0 | Yellow sand |
| 37A | 1.0-1.3 | Yellow sand |
| 37B | 2.7-3.0 | Brown sand |
| 38A | 1.5-1.8 | Grey sand |
| 39A | 1.0-1.3 | Yellow sand stones |
| 39B | 2.7-3.0 | Black stones |
| 40A | 1.7-2.3 | Yellow sand |
| 40B | 3.0-3.5 | Fine dark material and yellow sand |
| 40C | 4.0-4.5 | Organic material in yellow sand |
| 40D | 5.0-5.5 | Black coarse sand |
| 42A | 1.3-1.8 | Dark yellow sand and coal |
| 42B | 2.0-2.5 | Brown sand |
| 42C | 3.0-3.5 | Brown sand |
| 42D | 4.0-4.5 | Brown sand |
| 42E | 5.0-5.5 | Brown sand |

| Probe No. & Sample ID | Depth (m) | Description |
|---|---|---|
| 43A | 1.3-1.8 | Black yellow top soil |
| 43B | 2.0-2.5 | Yellow soil/ sand mixeture |
| 43C | 3.0-3.5 | Brown sand |
| 43D | 4.0-4.5 | Yellow sand |
| 43E | 5.0-5.5 | Brown sand |
| 44A | 1.3-1.8 | Yellow sand and organic matter |
| 44B | 2.0-2.5 | Brown sand |
| 44C | 3.0-3.5 | Yellow sand |
| 44D | 4.0-4.5 | Yellow sand |
| 44E | 5.0-5.5 | Dark brown sand and coal |
| 46A | 1.0-1.3 | Brown sand |
| 46B | 2.7-3.0 | Brown sand mixed with coal |
| 46C | 3.0-3.5 | Dark brown sand |
| 46D | 4.0-4.5 | Grey sand mixed with coal |
| 46E | 5.0-5.3 | Dark brown sand |
| 47A | 1.0-1.3 | Yellow clear sand |
| 47B | 2.0-2.3 | Yellow sand |
| 47C | 2.7-3.0 | Yellow sand |
| 47D | 3.0-3.5 | Brown sand |
| 47E | 4.0-4.5 | Brown sand |
| 47F | 5.0-5.5 | Pit soil |
| 47G | 3.0-5.5 | Black brown sand |
| 48A | ...-... | Blue brown sand |
| 48B | 2.7-3.0 | Yellow sand |
| 48C | 2.0-2.5 | Yellow sand |
| 48D | 3.0-3.5 | Yellow sand |
| 48E | 4.0-4.5 | Grey sand |
| 48F | 5.0-5.5 | 50 % coal and brown sand |
| 49A | 1.3-1.8 | Yellow sand |
| 49B | 2.0-2.5 | Yellow sand |
| 49C | 3.0-3.5 | Yellow sand |
| 49D | 4.0-4.5 | Yellow sand |
| 49E | 5.0-5.5 | Yellow sand and coal |
| 50A | 1.0-1.3 | Yellow sand |
| 50B | 1.3-1.6 | Yellow sand |
| 50C | 2.0-2.3 | Yellow sand |
| 50D | 4.0-4.3 | Brown sand |
| 50E | 5.0-5.3 | Brown sand |
| 50F | ...-... | Yellow sand |
| 60A | 1.0-1.5 | Grey sand |
| 60B | 2.7-3.0 | Fine grey black sand |
| 61A | 1.0-1.5 | Brown sand |
| 61B | 2.7-3.2 | Brown sand and coal |

5.2.2 Description of the fourth survey.

The fourth survey was conducted between late November and early December 1994 by taking eight Geoprobe cores. The samples were collected at 2 m spacing down to 8 metres depth. Ground water samples were also collected in labelled plastic bottles. All samples were brought to the Agricultural, Food and Environmental Chemistry Laboratory Glasgow University as soon as possible. The soil and water samples were stored in the deep freezer before analysis. The soil samples were analysed for ammonium and loss of ignition. The water samples were analysed for ammonium and pH. The probe hole number, bottle number and depth of each sample is given in Table 5.6 and Map 5.6 shows the locations of the sampling points.

Table 5.6 Soil sample identification for the fourth survey

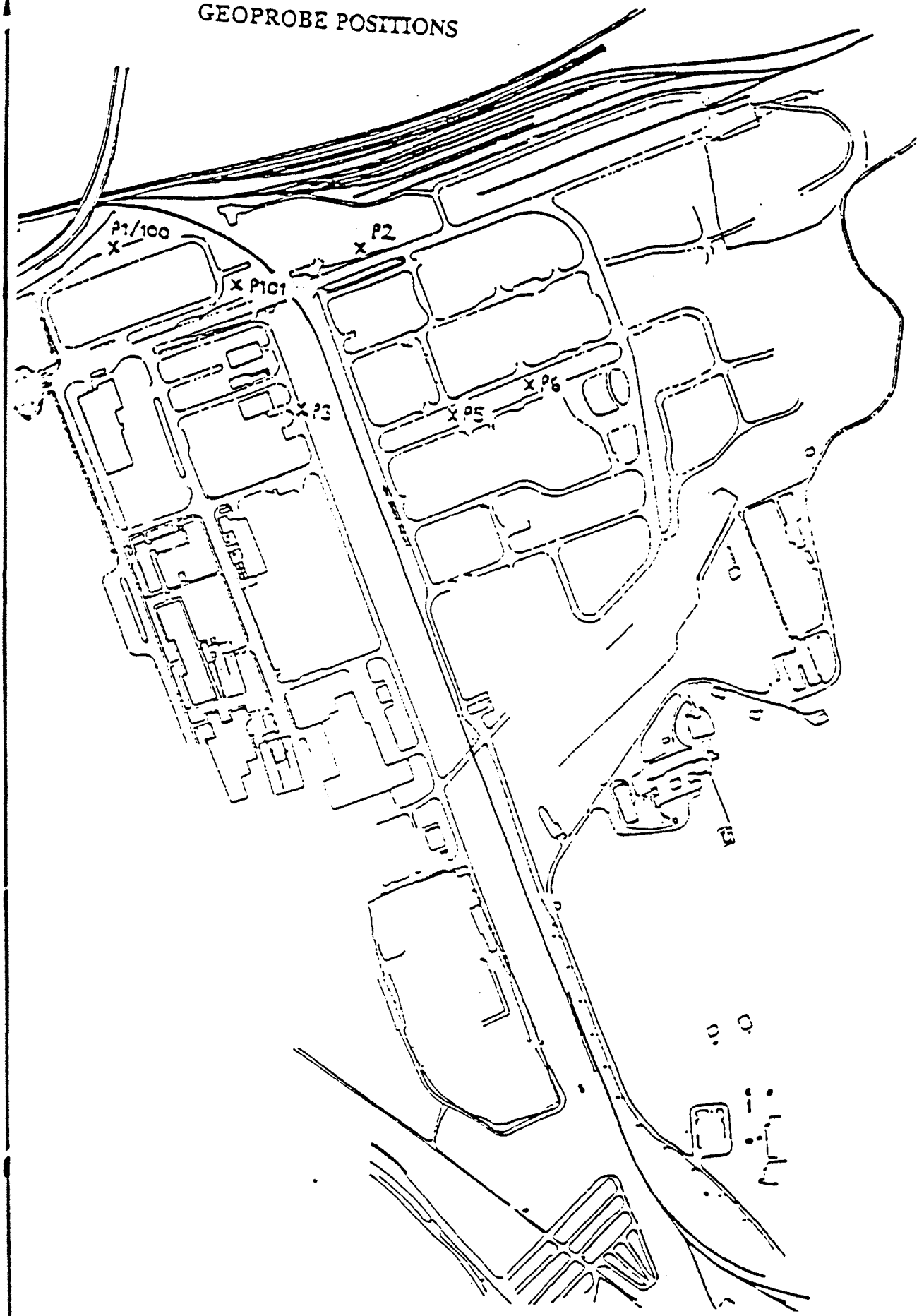
| Probe No | Date | Bottle No. | Depth (m) |
|---|---|---|---|
| P 1 | 14-12-94 | 032 | 2.0 |
| | | 037 | 4.0 |
| | | 046 | 6.0 |
| | | 047 | 8.0 |
| P 2 | 15-12-94 | 118 | 8.0 |
| P 3 | 15-12-94 | 095 | 2.0 |
| | | 100 | 4.0 |
| | | 109 | 6.0 |
| | | 110 | 8.0 |
| P 5 | 14-12-94 | 053 | 2.0 |
| | | 058 | 4.0 |
| | | 067 | 6.0 |
| | | 068 | 8.0 |
| P 6 | 14-12-94 | 074 | 2.0 |
| | | 079 | 4.0 |
| | 15-12-94 | 088 | 6.0 |
| | | 089 | 8.0 |
| 100 | 22-11-94 | 100 | 2.0 |
| | | 104 | 4.0 |
| | | 116 | 6.0 |
| | | 117 | 8.0 |
| 101 | 23-11-94 | 118 | 2.0 |
| | | 122 | 4.0 |
| | | 133 | 6.0 |
| | | 134 | 7.0 |
| | | 135 | 7.0 |
| 102 | 24-11-94 | 154 | 4.0 |
| | | 164 | 6.0 |
| | | 165 | 8.0 |

Table 5.7 Water sample identification for the fourth survey.

| Probe No | Date | Bottle No. | Depth (m) |
|---|---|---|---|
| P 1 | 14-12-94 | 044 | - |
| | | 048 | 8.0 |
| P 2 | 15-12-94 | 117 | 8.0 |
| P 3 | 15-12-94 | 107 | - |
| | | 111 | 8.0 |
| P 5 | 14-12-94 | 065 | 4.0 |
| | | 069 | 8.0 |
| P 6 | 14-12-94 | 086 | - |
| | | 090 | 8.0 |
| 100 | 22-11-94 | 112 | - |
| | | 147 | - |
| 101 | 23-11-94 | 130 | - |
| | | 140 | - |
| 102 | 24-11-94 | 161 | 4.5 |

Map 5.6 Sampling points of fourth survey.

GEOPROBE POSITIONS



## 5.2.2 Laboratory Analytical Methods

### 5.2.2.1 Routine analytical methods

The routine laboratory analytical methods for determining moisture content, organic matter content, pH, conductivity, mechanical analysis and cation exchange capacity of soil are described in Chapter 2 section 2.1.

### Determination of ammonium nitrogen in soil samples

The soil samples were extracted using 0.5 M potassium sulphate solution as discussed in Chapter 2 section 2.6. The extracts were analysed for measurement of ammonium nitrogen using the Technicon Autoanalyzer as in section 2.1.

### Determination of inorganic nitrogen, pH and conductivity in water samples.

Water samples were filtered through Whatman GF/C filter paper. The ammonium nitrogen was determined using the Technicon Auto-Analyser. The filtered water samples were also used for determination of nitrate nitrogen, pH and conductivity. The methods are described in Chapter 2.

## 5.3 RESULTS AND DISCUSSION

### 5.3.1 Results of the First Survey.

The concentrations of ammonium and nitrate, soil pH, moisture content and soil conductivity for the samples of the first survey are given in Table 5.8:

Table 5.8 Analytical results of soil samples of first survey.

| Sample No. | Depth (cm) | $NH_4$-N ( mg $kg^{-1}$ ) | $NO_3$-N ( mg $kg^{-1}$ ) | pH | %$H_2O$ | Conductivity uSiemens |
|---|---|---|---|---|---|---|
| 1A | 0-15 | 0.55 | 0.08 | 6.3 | 8.4 | 44 |
| 1B | 40-50 | 0.15 | 0.25 | 6.4 | 11.3 | 43 |
| 2A | 0-15 | 0.04 | 0.36 | 6.7 | 12.0 | 32 |
| 2B | 35-45 | 0.43 | 0.51 | 6.6 | 9.4 | 21 |
| 3A | 0-12 | 0.43 | 0.92 | 6.5 | 9.9 | 52 |
| 3B | 15-25 | 0.12 | 0.70 | 6.6 | 16.5 | 78 |
| 4A | 0-15 | 0.39 | 2.12 | 6.7 | 13.8 | 42 |
| 4B | 15-30 | 0.16 | 1.62 | 7.0 | 8.6 | 57 |
| 5A | 0-10 | 0.74 | 13.46 | 7.1 | 16.4 | 74 |
| 5B | 15-25 | 0.41 | 0.73 | 7.0 | 14.6 | 47 |

These soil samples of the A and B horizons of the soil profile were within, or close to the suspected contaminated area. Levels of soil ammonium-N and nitrate-N were within the range to be expected in unfertilized, semi-derelict soils with sparse vegetation and showed no evidence of contamination. The soil samples were obtained with a spade from shallow depths. After analysis it was proposed to take further samples from greater depth by JCB digger.

5.3.2 Results of the Second Survey.

The soil samples were obtained by digging trial pits with a JCB digger to depth of 3-4 metres depending on the depth of the water table. Samples down to 1 metre were taken by sampling from within the pits but the samples below this depth were taken from the JCB bucket. Difficulties in digging the trial pits and in obtaining samples from the pits at depths greater than 1 metre were seen due to collapsing of the sides of the pits because of the loose sandy subsoil. Therefore, only three trial pits were dug. The concentration of ammonium and nitrate, soil pH, soil conductivity and loss of ignition for the subsoil samples and water samples taken in second survey are presented in Tables 5.9 and 5.10 respectively.

Table 5.9 Analytical results for soil samples of the second survey.

| Sample ID | Depth (cm) | $NH_4$-N ( mg $kg^{-1}$ ) | $NO_3$-N | pH | moisture % | Conduct uS | LOI % |
|---|---|---|---|---|---|---|---|
| TP1A | 35-90 | 0.4 | 0.5 | 6.2 | 6.4 | 33 | 0.4 |
| TP1B | 91-155 | 15.5 | 0.8 | 5.8 | 23.7 | 44 | 11.3 |
| TP1C | 156-300 | 44.9 | 0.7 | 7.0 | 21.6 | 51 | 0.5 |
| TP1D | 301-350 | 23.4 | 0.9 | 6.8 | 27.5 | 43 | 0.4 |
| TP2A | 35-70 | 1.1 | 1.3 | 6.9 | 14.0 | 33 | 4.0 |
| TP2B | 71-100 | 0.9 | 0.4 | 7.4 | 7.1 | 65 | 0.7 |
| TP2C | 101-160 | 1.0 | 0.5 | 7.4 | 7.5 | 35 | 0.7 |
| TP2D | 161-180 | 1.1 | 0.3 | 7.4 | 5.5 | 32 | 0.4 |
| TP2E | 221-240 | 0.9 | 1.1 | 7.4 | 19.4 | 47 | 0.5 |
| TP2F | 350-400 | 0.9 | 2.7 | 7.5 | 24.3 | 79 | 0.2 |
| TP3A | 35-80 | 0.9 | 0.3 | 7.6 | 19.2 | 92 | 4.3 |
| TP3B | 81-120 | 1.1 | 0.7 | 7.8 | 15.3 | 88 | 1.2 |
| TP3C | 121-140 | 1.4 | 1.0 | 7.8 | 23.2 | 84 | 1.9 |
| TP3D | 141-160 | 1.6 | 1.2 | 7.8 | 25.5 | 90 | 1.4 |

Table 5.10 Analytical results of water samples of the second survey

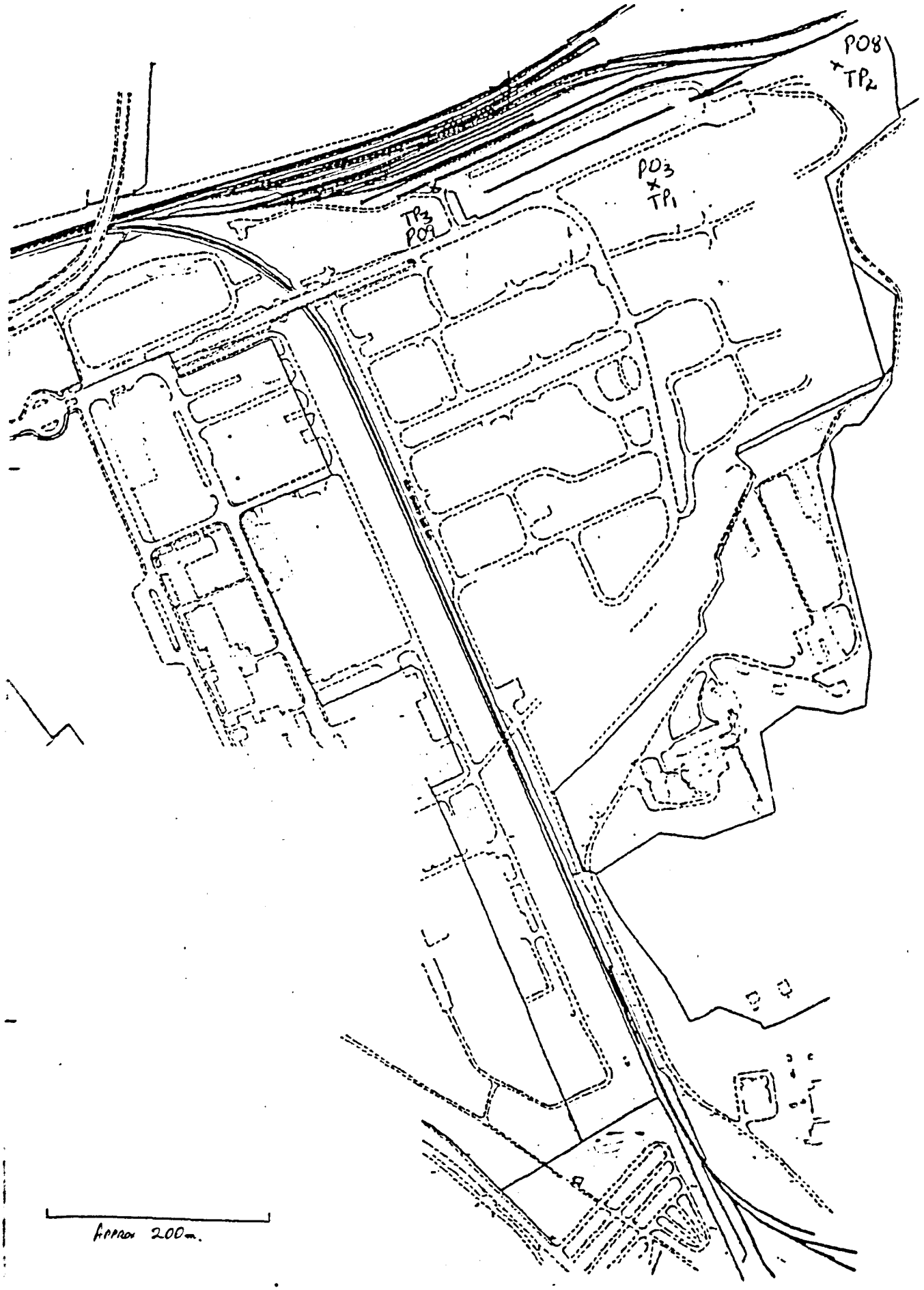
| Sample ID | Depth (cm) | $NH_4$-N ( mg $l^{-1}$ ) | $NO_3$-N | pH | Conductivity uSiemens |
|---|---|---|---|---|---|
| TP1CW | 156-300 | 57.70 | 0.05 | 8.3 | 548 |
| TP1DW | 301-350 | 22.50 | 0.03 | 8.2 | 409 |
| TP1GW | 301-350 | 39.00 | 0.02 | 8.4 | 603 |
| TP2FW | 350-400 | 0.04 | 4.10 | 7.7 | 884 |
| TP2GW | 350-400 | 0.04 | 3.10 | 7.6 | 806 |
| TP3CW | 121-140 | 0.02 | 0.66 | 7.6 | 332 |
| TP3DW | 141-160 | 0.02 | 0.51 | 7.2 | 326 |
| TP3GW | 160-200 | 0.04 | 0.25 | 7.2 | 295 |

Trial pit 1 does show some evidence of a peak of ammonium in the profile. Trial pits 2 and 3 do not show evidence of ammonium contamination even though TP3 was dug at the site of the previous ground water sample PO9 (December 1993) which gave a value of 90 mg $l^{-1}$ ammonium-N. Table 5.11 shows a comparison of the ammonium levels in water samples of this survey with the ground water samples taken at the same locations in the preliminary survey (dated December, 1993 ). Map 5.7 shows the position of the points.

Table 5.11 Matching of results of second survey, and boreholes from preliminary survey

| Trial Pit | | | Borehole | | |
|---|---|---|---|---|---|
| ID | Ammonium (mg $l^{-1}$) | pH | ID | Ammonium (mg $l^{-1}$) | pH |
| TP1 | 39.00 | 8.4 | PO3 | 5 | 6.9 |
| TP2 | 0.04 | 7.6 | PO8 | <1 | 7.3 |
| TP3 | 0.04 | 7.1 | PO9 | 90 | 7.7 |

Map 5.7 Comparison of sampling points of second and preliminary survey.


PO8
TP2
PO3
TP1
TP3
PO9
Approx 200m.

The reasons for these discrepancies may be the different sampling depths employed. Due to the difficulty in obtaining samples the picture is not clear. Ammonium was found in trial pit 1 and the highest ammonium value is seen at 1.5 to 3.0 metres. The level declines at the water table below 3.0 metres. This is also seen in the extracted pore water samples (see Table 5.10 ). If the ammonium is present as a more or less coherent band within the sand profile and to some extent associated with cation exchange sites rather than being freely soluble in the pore water; or if the ground water is stratified then the depth of sampling of either soil or ground water could be crucial to the detection of the ammonium.

Calculation of the proportion of the ammonium held on exchange sites or free in the pore solution ( based on the data in Tables 5.9 and 5.10 for trial pit 1) shows that approximately one quarter is free in solution (see Table 5.12).

Table 5.12 Partition of Ammonium between Exchange Sites and soil solution

| Sample ID | Depth | Ammonium-Nitrogen | | |
|---|---|---|---|---|
| | | Total | Exchangeable | Solution |
| | cm | ..... mg $kg^{-1}$ ..... | | |
| TP1C | 155-300 | 44.9 | 32.4 (72%) | 12.5 (28%) |
| TP1D | 300-330 | 23.4 | 17.2 (74%) | 6.2 (26%) |

To examine this further the samples were analysed for cation exchange capacity and exchangeable bases, loss on ignition and particle size analysis.

Table 5.13 Exchangeable Bases and Cation Exchange Capacity of second survey soil samples

| Sample ID | $Na^+$ | $K^+$ | $Ca^{++}$ | $Mg^{++}$ | $NH_4^+$ | Sum | CEC |
|---|---|---|---|---|---|---|---|
| | ---------- | | ($mmol_e\ kg^{-1}$) | | | ---------- | |
| TP1A | 2.1 | 0.5 | 9.5 | 0.4 | - | 12.5 | 14.9 |
| TP1B | 3.2 | 1.3 | 24.7 | 2.0 | - | 31.2 | 90.2 |
| TP1C | 2.9 | 0.4 | 4.6 | 0.5 | 2.3 | 10.7 | 11.1 |
| TP1D | 2.9 | 0.4 | 7.9 | 0.7 | 1.2 | 13.1 | 11.9 |
| TP2A | 3.9 | 0.6 | 47.3 | 1.0 | - | 52.8 | 39.8 |
| TP2B | 3.5 | 0.4 | 19.4 | 0.6 | - | 23.9 | 16.2 |
| TP2C | 2.7 | 0.4 | 13.3 | 0.4 | - | 16.8 | 13.5 |
| TP2D | 1.9 | 0.7 | 10.3 | 0.4 | - | 13.3 | 10.7 |
| TP2E | 2.3 | 0.4 | 8.3 | 0.3 | - | 11.3 | 10.7 |
| TP2F | 2.5 | 0.4 | 10.3 | 0.6 | - | 13.8 | 10.4 |
| TP3A | 2.5 | 1.2 | 60.4 | 1.7 | - | 65.8 | 61.3 |
| TP3B | 3.2 | 0.9 | 45.9 | 1.6 | - | 51.6 | 46.8 |
| TP3C | 3.7 | 0.8 | 44.5 | 1.7 | - | 50.7 | 43.6 |
| TP3D | 4.3 | 0.9 | 40.7 | 1.5 | - | 47.4 | 40.5 |

TABLE 5.14 Particle size analysis and loss on ignition of second survey soil samples.

| Sample ID | Coarse sand ( % ) | Fine sand ( % ) | Silt ( % ) | Clay ( % ) | Loss on ignition ( % ) |
|---|---|---|---|---|---|
| TP1A | 87.8 | 9.3 | 0.9 | 1.1 | 0.4 |
| TP1B | 67.4 | 13.7 | 12.9 | 8.7 | 11.3 |
| TP1C | 88.5 | 7.0 | 1.1 | 1.8 | 0.5 |
| TP1D | 89.9 | 8.7 | 1.2 | 2.1 | <0.1 |
| TP2A | 83.8 | 9.3 | 4.4 | 1 1 | 4.0 |
| TP2B | 90.2 | 5.9 | 1.0 | 1.8 | 0.8 |
| TP2C | 93.8 | 4.6 | 0.3 | 0.2 | 0.7 |
| TP2D | 90.3 | 8.0 | 0.8 | 0.5 | 0.4 |
| TP2E | 95.4 | 5.6 | 0.8 | 0.6 | 0.5 |
| TP2F | 93.3 | 4.8 | 1.5 | 0.7 | 0.2 |
| TP3A | 75.6 | 15.0 | 5.7 | 0.6 | 4.3 |
| TP3B | 81.0 | 9.6 | 3.0 | 2.2 | 1.2 |
| TP3C | 84.1 | 6.6 | 3.1 | 2.0 | 2.0 |
| TP3D | 81.0 | 12.5 | 3.7 | 1.2 | 1.4 |

Tables 5.13 and 5.14 show very low cation exchange capacities, and very low clay and organic matter contents. Values for the cation exchange capacity of up to 100 $mmol_e$ $kg^{-1}$ might be typical of a natural topsoil of the Dreghorn Soil series forming on this type of parent material.

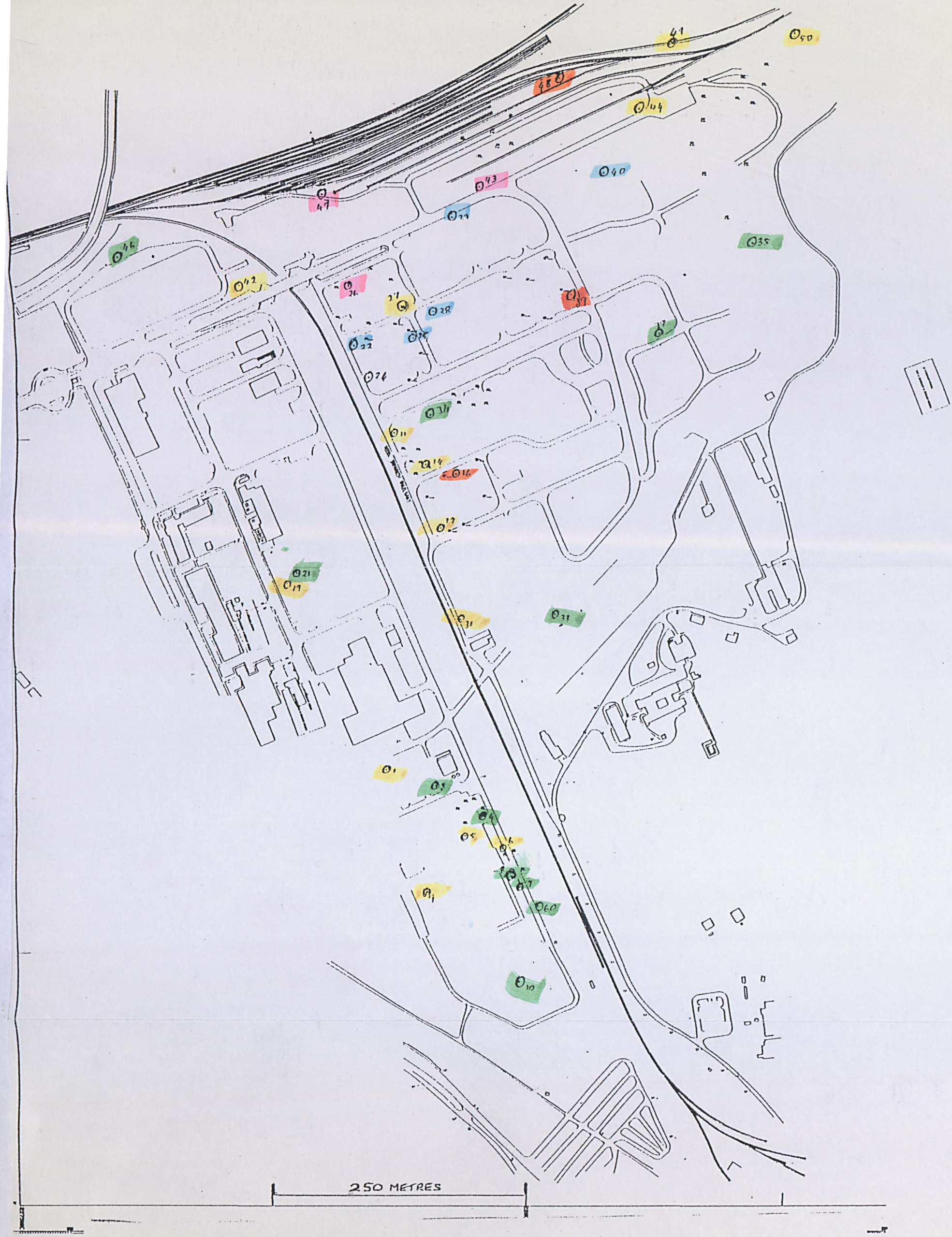
The cation exchange sites are dominated by calcium and in profiles 2 and 3 the sum of the exchangeable bases appears to exceed the exchange capacity. This may be due to the effect of the high pH of these two profiles. Firstly the CEC is measured at pH 7.0 and would be higher at higher pH values due to pH dependent cation exchange sites. Secondly the level of exchangeable calcium may be over estimated in soils containing calcium carbonate as traces may dissolve during the leaching of exchangeable cations.

In the lower part of profile 1 where high levels of ammonium were present, ammonium was a significant proportion of the exchangeable cations. This and the very low cation exchange capacity may account for the presence of significant levels of ammonium in solution.

### 5.3.3. Results of the Third Survey.

Due to the difficulties in sampling with trial pits and the greater depth of sampling required, ICI arranged for a geoprobe core survey to be carried out over the site as part of a wider assessment of contamination on the site. Samples of soil and water were collected for ammonium determination and analysis of relevent parameters for investigating ammonium contamination. There were also difficulties in obtaining an even spread of the sampling points due to concrete foundations on the site. Map 5.8 shows the ammonium level at 37 sampling points and Table 5.15 shows the ammonium and nitrate, pH, moisture content and loss of ignition of 115 soil samples collected from 37 probe holes. For all the 37 probe holes the ammonium concentration of the surface soil samples were within the range to be expected in unfertilized semi-derelict soils with sparse vegetation as discussed for the results of the first survey. The soil samples taken from below 2.5 metres show evidence of ammonium contamination. The levels of contamination varied from one probe hole to another.

Map 5.8 Ammonium levels of third survey.




mg $NH_4$-N per kg

| | | | |
|---|---|---|---|
| Normal | 0 | - | 5 |
| Slightly Elevated | 6 | - | 25 |
| Moderately Contaminated | 26 | - | 50 |
| Highly Contaminated | 51 | - | 100 |
| Extremely Contaminated | | > | 101 |

Table 5.15 Analytical results for the third survey of soil samples.

| Probe No. & Sample ID | Depth (m) | $NH_4$-N (ug/g) | $NO_3$-N | pH | $H_2O$ (%) | Cond (uS) | LOI (%) |
|---|---|---|---|---|---|---|---|
| 1A | 1.0-1.5 | 0.3 | 1.2 | 6.6 | 22.0 | 38 | 0.2 |
| 1B | 2.7-3.2 | 12.8 | 2.2 | 6.9 | 16.9 | 66 | 0.2 |
| 3A | 1.0-1.3 | 0.8 | 1.0 | 6.7 | 16.6 | 80 | 0.3 |
| 3B | 2.7-3.0 | 2.7 | 0.9 | 6.6 | 20.7 | 68 | 1.8 |
| 4A | 1.0-1.3 | 0.7 | 0.7 | 6.9 | 21.3 | 63 | 0.7 |
| 4B | 2.7-3.0 | 22.8 | 2.9 | 6.5 | 24.0 | 60 | 0.4 |
| 5A | 1.0-1.5 | 1.9 | 0.8 | 6.1 | 10.2 | 52 | 0.4 |
| 5B | 2.5-3.0 | 19.5 | 0.8 | 6.5 | 24.4 | 61 | 0.3 |
| 6A | 1.0-1.5 | 10.7 | 0.8 | 7.0 | 14.5 | 69 | 0.9 |
| 6B | 2.0-2.5 | 3.3 | 0.7 | 6.0 | 24.6 | 33 | 0.6 |
| 6C | 3.0-3.5 | 7.2 | 0.6 | 6.4 | 22.1 | 48 | 0.3 |
| 6D | 4.0-4.5 | 0.8 | 0.9 | 6.5 | 23.2 | 31 | 0.4 |
| 6E | 5.0-5.5 | 4.1 | 1.1 | 6.2 | 21.6 | 70 | 0.7 |
| 7A | 1.0-1.3 | 0.3 | 1.2 | 6.6 | 19.8 | 34 | 0.3 |
| 7B | 2.7-3.0 | 1.7 | 1.3 | 6.2 | 21.3 | 50 | 0.6 |
| 8A | 1.0-1.3 | 4.1 | 1.3 | 7.8 | 11.5 | 155 | 1.4 |
| 8B | 2.7-3.0 | 2.4 | 0.8 | 6.7 | 18.5 | 88 | 0.6 |
| 9A | 1.0-1.5 | 1.2 | 0.8 | 9.9 | 13.6 | 233 | 0.8 |
| 9B | 2.7-3.2 | 7.4 | 1.1 | 6.4 | 18.3 | 35 | 0.3 |
| 10A | 1.0-1.5 | 0.8 | 0.7 | 6.8 | 11.9 | 46 | 0.2 |
| 10B | 2.7-3.2 | 1.8 | 2.1 | 7.1 | 23.2 | 62 | 2.4 |
| 11A | 1.0-1.3 | 4.6 | 1.2 | 7.3 | 21.7 | 84 | 0.5 |
| 11B | 2.7-3.2 | 12.9 | 0.9 | 6.3 | 20.8 | 68 | 1.3 |
| 14A | 1.0-1.3 | 0.8 | 1.0 | 6.6 | 28.7 | 47 | 0.5 |
| 14B | 2.7-3.0 | 12.2 | 0.7 | 6.6 | 16.6 | 55 | 0.2 |
| 16A | 1.5-1.9 | 3.4 | 0.5 | 7.7 | 22.2 | 92 | 0.2 |
| 16B | 2.7-3.1 | 40.9 | 1.0 | 5.8 | 23.9 | 95 | 2.3 |
| 17A | 1.0-1.3 | 1.1 | 0.7 | 7.0 | 25.3 | 140 | 1.7 |
| 17B | 2.7-3.0 | 21.8 | 1.0 | 6.8 | 27.3 | 68 | 2.5 |
| 19A | 1.0-1.3 | 10.5 | 0.7 | 6.1 | 16.3 | 14 | 0.5 |
| 19B | 2.7-3.0 | 1.5 | 0.9 | 7.3 | 23.6 | 75 | 0.3 |
| 21A | 1.0-1.3 | 1.2 | 1.4 | 6.0 | 14.5 | 64 | 0.8 |
| 21B | 2.7-3.0 | 0.7 | 2.1 | 7.3 | 22.2 | 63 | 0.3 |
| 22A | 1.0-1.3 | 0.6 | 0.7 | 7.3 | 5.8 | 48 | 0.2 |
| 22B | 2.7-3.0 | 110.5 | 0.5 | 7.1 | 21.9 | 78 | 0.8 |

| Probe No. & Sample ID | Depth (m) | $NH_4$-N (ug/g) | $NO_3$-N | pH | $H_2O$ (%) | Cond (uS) | LOI (%) |
|---|---|---|---|---|---|---|---|
| 25A | 1.0-1.3 | 2.0 | 0.9 | 6.6 | 17.8 | 27 | 0.3 |
| 25B | 2.7-3.0 | 118.9 | 2.4 | 6.5 | 26.8 | 95 | 1.9 |
| 26A | 1.0-1.3 | 0.2 | 0.6 | 6.8 | 14.9 | 45 | 9.8 |
| 26B | 2.7-3.0 | 70.8 | 0.9 | 7.1 | 22.9 | 60 | 0.2 |
| 27A | 1.0-1.3 | 1.3 | 0.9 | 6.6 | 17.6 | 49 | 0.2 |
| 27B | 2.7-3.0 | 22.3 | 0.7 | 10.1 | 21.2 | 460 | 0.3 |
| 28A | 1.6-2.2 | 4.7 | 1.1 | 6.3 | 15.1 | 34 | 0.3 |
| 28B | 2.7-3.0 | 308.8 | 2.1 | 7.2 | 18.0 | 88 | 1.7 |
| 29A | 1.0-1.5 | 2.3 | 1.2 | 6.5 | 13.5 | 62 | 2.2 |
| 29B | 2.7-3.0 | 46.5 | 1.2 | 6.8 | 22.6 | 58 | 0.0 |
| 29C | 3.0-3.5 | 95.1 | 1.0 | 6.5 | 25.2 | 29 | 0.2 |
| 29D | 4.0-4.5 | 257.6 | 0.9 | 7.0 | 25.6 | 63 | 1.2 |
| 29E | 5.0-5.5 | 234.9 | 1.0 | 7.1 | 24.7 | 79 | 0.7 |
| 31A | 1.0-1.7 | 0.6 | 0.8 | 7.3 | 15.9 | 68 | 0.5 |
| 31B | 2.7-3.0 | 5.6 | 0.9 | 6.3 | 20.7 | 42 | 0.4 |
| 33A | 1.0-1.3 | 1.0 | 1.3 | 7.9 | 11.6 | 80 | 1.5 |
| 33B | 2.0-2.3 | 1.3 | 0.5 | 6.9 | 23.7 | 71 | 1.2 |
| 33C | 2.7-3.0 | 1.8 | 0.6 | 7.4 | 17.0 | 82 | 0.4 |
| 33D | 3.0-3.3 | 4.0 | 0.8 | 6.7 | 22.5 | 32 | 0.5 |
| 33E | 4.0-4.3 | 1.7 | 0.8 | 6.1 | 21.4 | 109 | 1.4 |
| 33F | 5.0-5.3 | 3.1 | 0.8 | 6.5 | 24.3 | 110 | 1.5 |
| 34A | 1.0-1.3 | 0.5 | 0.7 | 6.6 | 5.4 | 52 | 0.4 |
| 34B | 2.7-3.0 | 1.5 | 1.1 | 6.8 | 18.4 | 96 | 0.5 |
| 37A | 1.0-1.3 | 1.5 | 0.6 | 6.5 | 22.4 | 62 | 0.3 |
| 37B | 2.7-3.0 | 2.1 | 1.1 | 7.1 | 22.9 | 72 | 0.4 |
| 38A | 1.5-1.8 | 0.4 | 0.8 | 6.7 | 13.2 | 72 | 0.2 |
| 39A | 1.0-1.3 | 1.0 | 0.6 | 6.3 | 13.2 | 47 | 0.5 |
| 39B | 2.7-3.0 | 46.4 | 1.4 | 7.0 | 17.8 | 107 | 0.5 |
| 40A | 1.7-2.3 | 0.8 | 1.9 | 6.6 | 3.9 | 68 | 0.1 |
| 40B | 3.0-3.5 | 7.8 | 0.7 | 6.6 | 25.5 | 49 | 0.5 |
| 40C | 4.0-4.5 | 114.2 | 0.7 | 5.6 | 26.4 | 124 | 2.2 |
| 40D | 5.0-5.5 | 192.3 | 2.4 | 7.7 | 20.5 | 97 | 0.3 |
| 42A | 1.3-1.8 | 2.1 | 0.6 | 6.9 | 11.6 | 68 | 1.4 |
| 42B | 2.0-2.5 | 0.7 | 1.2 | 6.6 | 23.9 | 29 | 0.4 |
| 42C | 3.0-3.5 | 6.3 | 1.2 | 6.4 | 22.6 | 30 | 0.3 |
| 42D | 4.0-4.5 | 0.7 | 1.0 | 7.2 | 22.5 | 75 | 0.2 |
| 42E | 5.0-5.5 | 7.9 | 0.6 | 6.7 | 24.5 | 70 | 1.1 |

| Probe No. & Sample ID | Depth (m) | $NH_4$-N (ug/g) | $NO_3$-N | pH | $H_2O$ (%) | Cond (uS) | LOI (%) |
|---|---|---|---|---|---|---|---|
| 43A | 1.3-1.8 | 1.1 | 0.8 | 7.4 | 6.2 | 53 | 0.4 |
| 43B | 2.0-2.5 | 1.4 | 1.3 | 7.2 | 22.9 | 569 | 0.7 |
| 43C | 3.0-3.5 | 17.3 | 0.7 | 7.7 | 21.2 | 92 | 0.3 |
| 43D | 4.0-4.5 | 49.5 | 1.0 | 7.0 | 23.7 | 45 | 0.3 |
| 43E | 5.0-5.5 | 76.0 | 0.8 | 6.7 | 19.7 | 64 | 0.3 |
| 44A | 1.3-1.8 | 1.7 | 0.6 | 7.0 | 7.9 | 100 | 0.7 |
| 44B | 2.0-2.5 | 1.2 | 0.7 | 8.6 | 7.8 | 96 | 0.7 |
| 44C | 3.0-3.5 | 0.5 | 1.0 | 6.7 | 10.6 | 45 | 0.2 |
| 44D | 4.0-4.5 | 2.6 | 2.2 | 7.2 | 22.7 | 80 | 0.3 |
| 44E | 5.0-5.5 | 11.0 | 0.9 | 6.3 | 20.9 | 93 | 1.2 |
| 46A | 1.0-1.3 | 1.7 | 0.5 | 7.3 | 8.6 | 39 | 1.5 |
| 46B | 2.7-3.0 | 1.1 | 1.1 | 7.7 | 20.3 | 180 | 2.6 |
| 46C | 3.0-3.5 | 2.7 | 1.6 | 7.8 | 12.1 | 127 | 3.0 |
| 46D | 4.0-4.5 | 1.4 | 1.0 | 6.6 | 24.1 | 70 | 0.2 |
| 46E | 5.0-5.3 | 2.5 | 0.6 | 6.9 | 22.7 | 72 | 0.3 |
| 47A | 1.0-1.3 | 0.7 | 0.7 | 8.0 | 4.7 | 105 | 0.3 |
| 47B | 2.0-2.3 | 0.6 | 3.1 | 6.5 | 23.5 | 55 | 0.1 |
| 47C | 2.7-3.0 | 22.3 | 0.6 | 8.0 | 24.4 | 95 | 0.2 |
| 47D | 3.0-3.5 | 8.9 | 1.0 | 6.7 | 23.9 | 58 | 0.2 |
| 47E | 4.0-4.5 | 64.9 | 0.6 | 7.0 | 24.7 | 55 | 0.5 |
| 47F | 5.0-5.5p | 6.4 | 1.3 | 4.3 | 87.6 | 1252 | 16.4 |
| 47G | 5.0-5.5s | 69.5 | 1.7 | 6.3 | 28.0 | 130 | 0.9 |
| 48A | 1.0-1.3 | 0.7 | 0.6 | 7.8 | 11.2 | 137 | 1.8 |
| 48B | 2.7-3.0 | 0.9 | 0.8 | 6.8 | 5.4 | 54 | 0.4 |
| 48C | 2.0-2.5 | 1.4 | 0.6 | 6.2 | 4.0 | 20 | 0.4 |
| 48D | 3.0-3.5 | 1.8 | 1.3 | 7.0 | 21.6 | 100 | 0.2 |
| 48E | 4.0-4.5 | 1.5 | 0.8 | 6.8 | 22.1 | 80 | 0.3 |
| 48F | 5.0-5.5 | 47.5 | 1.0 | 6.6 | 40.6 | 50 | 3.1 |
| 49A | 1.3-1.8 | 1.6 | 0.9 | 6.5 | 4.6 | 46 | 0.3 |
| 49B | 2.0-2.5 | 0.6 | 0.4 | 6.6 | 1.3 | 71 | 0.3 |
| 49C | 3.0-3.5 | 1.6 | 0.9 | 6.7 | 16.7 | 94 | 0.3 |
| 49D | 4.0-4.5 | 2.4 | 1.2 | 6.7 | 23.1 | 45 | 0.3 |
| 49E | 5.0-5.5 | 18.6 | 1.8 | 6.1 | 23.5 | 125 | 1.0 |
| 50A | 1.0-1.3 | 2.4 | 1.1 | 7.5 | 4.1 | 70 | 0.5 |
| 50B | 1.3-1.6 | 0.8 | 0.6 | 6.6 | 5.7 | 53 | 0.3 |
| 50C | 2.0-2.3 | 2.2 | 0.5 | 6.2 | 4.9 | 55 | 0.3 |
| 50D | 3.0-3.3 | 1.0 | 0.5 | 6.6 | 23.4 | 65 | 0.1 |
| 50E | 4.0-4.3 | 1.4 | 0.9 | 6.4 | 24.9 | 65 | 0.2 |
| 50F | 5.0-5.3 | 6.1 | 0.7 | 6.4 | 28.7 | 95 | 0.7 |
| 60A | 1.0-1.5 | 0.6 | 0.8 | 8.3 | 25.2 | 90 | 0.0 |
| 60B | 2.7-3.0 | 0.6 | 0.7 | 6.5 | 21.3 | 60 | 1.3 |
| 61A | 1.0-1.5 | 0.9 | 0.6 | 6.2 | 14.4 | 18 | 0.1 |
| 61B | 2.7-3.2 | 0.8 | 1.3 | 6.5 | 23.2 | 60 | 0.7 |

Table 5.16 shows the results of analysis of the water samples which were collected during the third survey for ammonium determination and the analysis of relevent parameters for investigating ammonium contamination.

Table 5.16 Analytical result of water samples for the third survey.

| Probe No. | depth (m) | $NH_4$-N (mg/l) | pH | Conductivity (uS) |
|---|---|---|---|---|
| 1 | 3.5 | 3 | 6.0 | 339 |
| 3 | 2.5 | <1 | 6.6 | 286 |
| 4 | 2.5 | 6 | 6.8 | 540 |
| 5 | 3.4 | 6 | 7.5 | 524 |
| 6 | 3.4 | 1 | 6.0 | 298 |
| 7 | 2.5 | <1 | 6.6 | 174 |
| 8 | 2.5 | <1 | 7.0 | 438 |
| 9 | 3.5 | 2 | 6.0 | 514 |
| 10 | 3.4 | <1 | 6.0 | 373 |
| 11 | 2.5 | <1 | 7.0 | 445 |
| 14 | 3.4 | <1 | 7.3 | 424 |
| 16 | 2.7 | 1 | - | 697 |
| 17 | 2.8 | 1 | 9.1 | 758 |
| 19 | 2.5 | <1 | 6.9 | 589 |
| 21 | 2.5 | <1 | 8.1 | 584 |
| 22 | 2.4 | 20 | 7.6 | 730 |
| 25 | 2.5 | 5 | 6.7 | 264 |
| 26 | 2.5 | <1 | 7.3 | 638 |
| 27 | 2.5 | <1 | 7.4 | 418 |
| 28 | 2.5 | 72 | 7.8 | 968 |
| 29 | 2.5 | 14 | 7.8 | 306 |
| 31 | 2.4 | <1 | 6.6 | 515 |
| 33 | 3.8 | <1 | 6.9 | 927 |

| Probe No. | depth (m) | $NH_4$-N (mg/l) | pH | Conductivity (uS) |
|---|---|---|---|---|
| 34 | 4.0 | <1 | 6.7 | 615 |
| 37 | 2.5 | <1 | 7.0 | 376 |
| 38 | 2.5 | <1 | 6.7 | 669 |
| 39 | 3.0 | 40 | 7.5 | 528 |
| 40 | 3.5 | 4 | 6.7 | 366 |
| 42 | 3.0 | 2 | 7.1 | 445 |
| 43 | 4.0 | 10 | 7.4 | 555 |
| 44 | 4.0 | 1 | 7.0 | 367 |
| 46 | 5.0 | <1 | 6.7 | 822 |
| 47 | 2.5 | <1 | 7.2 | 541 |
| 48 | 4.5 | <1 | 6.8 | 423 |
| 49 | 4.0 | 3 | 7.3 | 368 |
| 50 | 3.8 | <1 | 7.5 | 466 |
| 60 | 3.4 | <1 | 6.0 | 369 |
| 61 | 3.4 | <1 | 6.0 | 199 |

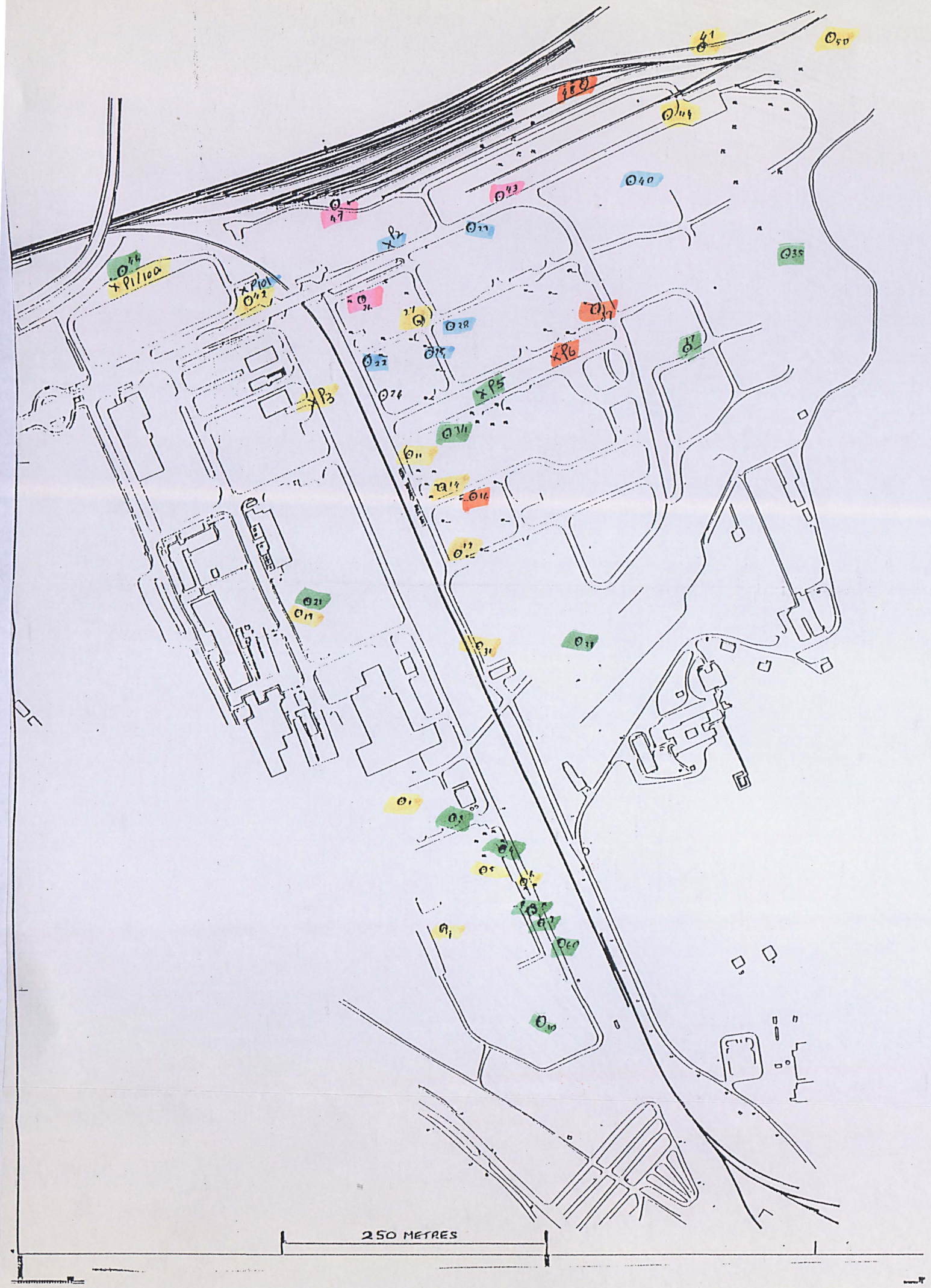
In the areas where ammonium contamination was found, the contamination level increased with depth, however many of the geoprobe boreholes may not have been deep enough to reveal the full extent of ammonium contamintion. Heavy contamination was localised to the part of the site close to where liquid ammonia was delivered by rail, transferred to a storage tank and utilised in the factory processing as shown in Map 5.8. Low measured levels of nitrate indicate no nitrification of ammonium. Generally low levels of loss on ignition were measured but in some samples a band with a

high percentage of organic matter was measured. Soil pH was in the range of 5.6 -10.0 but showed no relationship with increased ammonium contamination. Similarly soil conductivity did not show a relation with contamination.

5.3.4. Results of the fourth survey.

The fourth survey was conducted to measure ammonium concentrations to greater depths and to fill in some of the gaps in sampling for the third survey. The ammonium concentrations measured in soil and water samples for the fourth survey are presented in Tables 5.17 and 5.18. Map 5.9 shows the ammonium concentration at all the sampling points for both survey 3 and survey 4.

Map 5.9 Ammonium levels of third and fourth surveys.




mg $NH_4$-N per kg

| | |
|---|---|
| Normal | 0 - 5 |
| Slightly Elevated | 6 - 25 |
| Moderately Contaminated | 26 - 50 |
| Highly Contaminated | 51 - 100 |
| Extremely Contaminated | > 101 |

Table 5.17 Ammonium content and percentage loss on ignition of soil samples from the fourth survey.

| Probe No | Bottle No. | Depth (m) | $NH_4$ -N (mg $kg^{-1}$) | LOI (%) |
|---|---|---|---|---|
| P 1 | 032 | 2.0 | 0.23 | 0.37 |
| | 037 | 4.0 | 0.98 | 0.35 |
| | 046 | 6.0 | 6.00 | 0.33 |
| | 047 | 8.0 | 8.10 | 1.91 |
| P 2 | 118 | 8.0 | 405.00 | 1.47 |
| P 3 | 095 | 2.0 | 0.50 | 0.38 |
| | 100 | 4.0 | 18.90 | 1.23 |
| | 109 | 6.0 | 14.90 | 1.68 |
| | 110 | 8.0 | 10.90 | 11.46 |
| P 5 | 053 | 2.0 | 3.20 | 0.40 |
| | 058 | 4.0 | 6.90 | 0.57 |
| | 067 | 6.0 | 5.00 | 0.59 |
| | 068 | 8.0 | 3.10 | 6.77 |
| P 6 | 074 | 2.0 | 1.50 | 0.34 |
| | 079 | 4.0 | 3.10 | 0.69 |
| | 088 | 6.0 | 17.40 | 2.09 |
| | 089 | 8.0 | 29.60 | 2.05 |
| 100 | 100 | 2.0 | 2.10 | 0.39 |
| | 104 | 4.0 | 50.00 | 0.90 |
| | 116 | 6.0 | 83.20 | 1.16 |
| | 117 | 8.0 | 10.40 | 0.81 |
| 101 | 118 | 2.0 | 20.30 | 0.40 |
| | 122 | 4.0 | 154.00 | 1.61 |
| | 133 | 6.0 | 274.00 | 3.89 |
| | 134 | 7.0 | 496.00 | 3.11 |
| | 135 | 7.0 | 209.00 | 0.67 |
| 102 | 154 | 4.0 | 108.00 | 0.84 |
| | 164 | 6.0 | 32.20 | 0.80 |
| | 165 | 8.0 | 18.40 | 0.81 |

Table 5.18 Ammonium content and pH of water samples from the fourth survey.

| Probe No | Bottle No. | Depth (m) | $NH_4$-N (mg $l^{-1}$) | pH |
|---|---|---|---|---|
| P 1 | 044 | 4.0 | 0.06 | 6.3 |
| | 048 | 8.0 | 2.80 | 1.8 |
| P 2 | 117 | 8.0 | 519.00 | 9.9 |
| P 3 | 107 | 4.0 | 0.04 | 6.7 |
| | 111 | 8.0 | 4.00 | 6.4 |
| P 5 | 065 | 4.0 | 2.80 | 6.7 |
| | 069 | 8.0 | 1.40 | 6.4 |
| P 6 | 086 | 4.0 | 22.90 | 6.5 |
| | 090 | 8.0 | 56.90 | 5.5 |
| 100 | 112 | 4.0 | 40.30 | 7.1 |
| | 147 | 8.5 | 8.90 | 7.0 |
| 101 | 130 | 4.0 | 140.00 | 7.3 |
| | 140 | 7.0 | 424.00 | 9.9 |
| 102 | 161 | 4.5 | 66.30 | 8.0 |

The ammonium concentrations in soil and water samples for the fourth survey also show the same trend of contamination as in the third survey. High ammonium levels, up to 519 mg $l^{-1}$ were found in water samples and up to 496 mg $kg^{-1}$ in the soil samples. In addition a high pH of 9.9 was measured in two water samples having high ammonium contamination and a striking ammonia odour from 8 metre depth.

The ammonium content of 2.3 $mmol_e$ $kg^{-1}$ on the exchange sites shows that it is a significant proportion (21 %) of the exchangeable cations. The proportion of ammonium held on exchange sites or free in the pore solution shows that approximately one quarter of the

ammonium was free in the soil solution. The factory was closed down in 1980, but even after the passing of 14 years most of the ammonium is still present at the place where it was utilised. This is surprising and suggests that the ammonium will continue to leach down for many years.

It is suggested that the possible sources of ammonium contamination are:-

1. The spillage of ammonia on the surface.
2. Corrosion of an underground ammonia storage tank.
3. Contaminated mine drainage

If it is considered that the contamination is due to the corrosion of a tank, then recent corrosion could account for the presence of high levels of ammonium in ground water. The corrosion of an abandoned underground storage tank is discounted as there are no records of underground storage tanks and pipes at the site. The third reason, ammonium contamination from mine drainage, is not likely due to the very high levels of ammonium measured and the fact that the contamination is localised. Despite the finding of high levels of ammonium in solution at depths below the water table after the passage of 14 years, the most likely reason for the contamination is surface spillage during the factory operation period.

In order to determine the full extent of the ammonium contamination it would be necessary to conduct a systematic survey of ammonium contamination by collecting a large number of samples and digging boreholes down to at least 10 metres depth where the hard carboniferous sandstone and boulder clay strata start.

# CHAPTER 6

## GENERAL CONCLUSIONS

The role of this chapter is to draw together the main findings and conclusions of these studies. Since there is not enough time to continue the study for many of the aspects covered, some suggestions for further investigation are also made.

The studies carried out have been concerned with the evaluation of Kjeldahl digestion methods and the reactions of applied ammonical fertiliser in different soils with different moisture and temperature conditions. As well a site investigation of ammonium contamination was carried out at a former nylon factory area.

### 6.1 EVALUATION OF KJELDAHL DIGESTION METHOD

The Kjeldahl digestion method is the most widely used method for determining total nitrogen in plant and soil samples (Nelson and Sommers 1980, Bremner and Mulvaney 1982, Morries 1983, Preeze and Bate 1989, Jensen 1991, Tel and Jansen 1992 and Eric et al. 1993).

The method consists of two main steps, digestion and determination. The digestion step is influenced by salt concentration, catalyst, acid volume, temperature, sample weight, sample pretreatment, time of digestion and the ratios among these factors (Nelson and Sommers 1980, Bremner and Mulvaney 1982).

This evaluation of the Kjeldahl digestion procedure has been carried out by digesting certified reference plant materials of hay and cabbage and two soil samples. The influence of salt content, catalyst mixtures and the most

common pretreatment of salicylic acid on the standard Kjeldahl method were studied. The effects of sodium sulphate and potassium sulphate salts along with copper sulphate and selenium catalysts, with and without pre-treatment by the salicylic acid modification on the standard Kjeldahl method, was also investigated by using three treatments of salt/catalyst mixtures, 1 g or 2.5 g of 100:10 sodium sulphate and copper sulphate and 2.5 g of 100:10:1 potassium sulphate, copper sulphate and selenium.

The standard Kjeldahl digestion method with 1 g of sodium sulphate/copper sulphate mixture (100:10) measured significantly lower nitrogen ($P<5\%$) than the certified reference value for hay but not cabbage. Significantly lower ($P<5\%$) total nitrogen was also measured in soil samples with 1 g catalyst mixture in the standard Kjeldahl digestion method compared with 2.5 g of sodium sulphate/ copper sulphate mixture (100:10) and Kjeltabs (2.5 g potassium sulphate/copper sulphate/selenium, 100:10:1). The reason for the lower recovery of total nitrogen with 1 g of catalyst in the standard Kjeldahl digestion method is the lower digestion temperature which causes incomplete digestion (Bremner, 1960).

The 2.5 g of catalyst mixture and Kjeltabs used with the standard method gave significantly ($P<5\%$) higher total nitrogen recovery than the certified values for both hay and cabbage. The high values of total nitrogen measured for plant material were probably due to: i) Variable recovery of the high levels of nitrate which were present in the plant material at 3.1 mg $g^{-1}$ (hay) and 3.2 mg $g^{-1}$

(cabbage). ii) The method ( Hydrogen peroxide method) used by Commission of European Communities, Community Bureau of Reference for determination of total nitrogen for hay powder would be expected to give a lower recovery of total nitrogen than the standard Kjeldahl method used (Nelson and Sommers 1973 and Singh 1984).

The salicylic acid modification method measured significantly higher total nitrogen than the certified reference values with all catalyst mixtures for both plant materials. This higher recovery was due to partial recovery of nitrate as the reference method would not have recovered nitrate. Nitrate recovery was incomplete (35-75%) by the salicylic acid modification method. The salicylic acid modification method recovered significantly lower total nitrogen from soil samples than the standard Kjeldahl method. The soil samples contained insignificant levels of nitrate and the salicylic acid modification method results show nitrogen losses, particularly in the treatment using Kjeltabs which contain selenium.

The nitrate content in the Dreghorn soil is higher than Midelney soil series that is 0.057 mg $g^{-1}$ (Dreghorn) and 0.037 mg $g^{-1}$ (Midelney). It is difficult to see whether the salicylic acid modification method is recovering nitrate nitrogen or not as the total nitrogen recovered values are lower than with standard Kjeldahl method and the standard deviation of the total nitrogen measurments is higher than the nitrate content in the soils.

In general it is concluded that the standard Kjeldahl method with 2.5 g of sodium sulphate and copper sulphate

(100:10) or Kjeltab is better than the salicylic acid modification method for analysis of soils, which contain low levels of nitrate. This method gave a higher recovery of total nitrogen than the salicylic acid modification method as well as saving time, labour and reagents.

The determination step uses different techniques for determining ammonium concentration in the digest, such as steam distillation, colorimetric methods, or selective ion electrode methods (Eric et al. 1993). These methods have different degrees of sophistication, sensitivity, cost and speed. The selective ion electrode method is more costly compared to the steam distillation but not compared with the Technicon Autoanalyser method. The Technicon Autoanalyser method is more rapid than the other two methods. The selective ion electrode method requires continual restandardization, and is not as sensitive as distillation or colorimetric procedures. In this experiment a comparison of two methods for determining ammonium in digests by steam distillation and an automated colorimetric method using the Technicon Autoanalyzer was also made.

The comparison of the steam distillation method and the Technicon Autoanalyser method showed a nonsignificant difference of measured values of ammonium in digests of both plant materials ( hay and cabbage ) and Midelney and Dreghorn soil samples. No interference effect of selenium or copper was found in the colorimetric analysis of ammonium nitrogen. The Technicon Auto-Analyser method can automatically analyse 40 samples per hour including diluting and neutralising the digest while the steam

distillation titration method has a greater labour requirement. One analyst can analyse up to 12 samples per hour on a single distillation unit, but greater rates can be achieved using semi automated distillation systems.

The possible use of Kjeldahl digests for multielement analysis was investigated by analysing for phosphorus by the Ascorbic/Molybdate method and the Molybdate/ Metavanadate method on the Technicon Auto-Analyser and potassium by flame photometer in the digests of the certified reference materials. Thomas et al. (1967), Van lierop (1976), Novosamsky (1983) and Armando and Nelson (1991) have used Kjeldahl digestion for multielement analysis for plant materials. The main advantages of using Kjeldahl digestion for multielement analysis are to save time, labour, reagents and reduce the cost of analysis.

The measured values of phosphorus and potassium in the reference plant materials were compared with the certified values. The effect of two digestion methods with three salt catalyst mixtures on digestion was compared and comparison of two methods of determination of phosphorus was also made.

The phosphorus recovery from plant material of hay showed little effect of catalyst mixture by both methods of digestion while nonsignificant effects of catalysts and digestion methods were found in cabbage. The measured values of phosphorus were significantly greater ($P<5\%$) than the certified values for both hay and cabbage samples.

The comparison of the Ascorbic acid /Molybdate method and the Metavanadate/ Molybdate method for phosphorus

determination shows that the two methods were significantly different (P<5%). The Ascorbic acid/Molybdate method measured significantly lower phosphorus than the Metavanadate/ Molybdate method. The comparison of the effects of salt catalyst mixture indicated that there is no interference effect of selenium or copper on the colorimetric analysis of phosphorus by both methods. The amount of phosphorus recovered by the Ascorbic acid/ Molybdate method was more accurate than by the Molybdate/ Metavanadate method on the Technicon Auto-Analyser but the recovered values were still significantly higher than the certified values. For more accuracy and precision the analytical methods of phosphorous determination by Kjeldahl digestion method needs further improvements.

The Kjeltabs which contain potassium sulphate are not suitable for digestion of samples for measuring potassium. The standard Kjeldahl method with both 1 g and 2.5 g of sodium sulphate/ copper sulphate catalyst mixture recovered significantly lower potassium than the certified values for both plant materials. The salicylic acid modification with 1 g of catalyst mixture recovered significantly higher (P<5%) potassium than the certified values for both plant materials. The recovery using the salicylic acid modification method with 2.5 g of catalyst mixture was not significantly different to the certified value for hay but was significantly lower (P<5%) than the certified value for cabbage.

The use of these Kjeldahl digestion methods was not considered suitable for multielement analysis due to the

variable effects of salt and catalyst on the recovery of phosphorus and potassium.

## 6.2 NITRIFICATION

In addition to plant uptake, applied ammonium fertilisers are subject to losses by ammonia volatilisation, immobilisation by microorganisms, and fixation by clay. Additional losses occur following oxidation of ammonium to nitrite and nitrate by chemoautotrophic bacteria. Nitrate is rapidly leached and denitrified under anaerobic conditions. The rate of ammonium transformation is effected by soil moisture conditions, temperature, soil pH, cropping and their interaction. The study of applied ammonium nitrogen transformations at different moisture conditions, temperatures and pH under laboratory conditions provides models for predicting the fate of ammonium in soil following application of fertiliser. In the present study a comparison of the rates of ammonium disappearance and nitrate formation of five air dried and fresh soil samples has been made. The effect of low temperature on nitrification and mineralisation has been examined for four soils. The ammonium disappearance rates with repeated application of ammonium have been also compared for five soils.

In general it was concluded that the ammonium disappearance and nitrate formation rates were faster in fresh soils samples than in the air dried samples for the five soils tested. The air dried soil samples also showed a

short lag period (2 days) in ammonium disappearance and nitrate formation whereas the fresh samples lacked this lag period. The nitrate formation curves for the air dried soils samples appeared to be sigmoid curves while for fresh soils, ammonium disappearance and nitrate formation fitted a straight line and zero order rate constants were calculated for the nitrification rates. Anderson et al. (1969), Myers (1974), Seifert (1980) and Yousaf (1985) used air dried soil for determining ammonium nitrification at different temperatures. In general their papers show long incubation pericds (6-8 weeks), wide sampling intervals (1-2 weeks), and slow ammonium disappearance and nitrate formation. Great variability is seen in measured values and the lack of sufficient points makes it difficult to draw lines and to see the shape of the nitrification curves. Addiscott (1983), Flowers and O'Callaghan (1982) and Muzumdar (1992) used fresh soils and Shah (1988) used fresh coal mine spoils for measuring nitrification rates. Their results show short incubation periods, more frequent sampling intervals, very low variability, and clearly defined lines indicating no lag period.

In this experiment nitrification rates were calculated by two ways from the linear part of the graphs of the nitrate formation and ammonium disappearance. The nitrate formation rates were slightly higher compared to ammonium disappearance rates in the five soils due to mineralisation.

The ammonium disappearance and nitrate formation rates were significantly higher in the fresh subsamples

than in the air dried subsamples of soils. The reason for slower nitrification rates in air dried soils is reduction in the population of Nitrosomonas and Nitrobacter (Mortenson and Duley, 1931).

The mineralisation rates were calculated by regressing the linear parts of total inorganic nitrogen graphs. The mineralisation rates were higher in the air dried subsamples than fresh subsamples of all the soils. The reason for the increased mineralisation rates in air dried soil is the death of a more general population of microorganisms, decomposition of organic matter and the disturbance of soil structure. Air drying and storage of soils are known to affect soil properties ( Birch, 1958) and microorganisms (Ross and Niel, 1975).

The effect of low temperature (0, 5, 10, 15 and 20 °C) on nitrification was calculated from ammonium disappearance rates and nitrate formation rates. The ammonium disappearance and nitrate formation rates increased from 0 °C to 20 °C in all soils tested. There was a break in the temperature response curves at above 10 °C in these soils. The $Q_{10}$ values of ammonium disappearance and nitrate formation rates were similar in all four soils. The $Q_{10}$ values increased with decrease in temperature. The $Q_{10}$ values above 10 °C were near 2.0 while below 10 °C were more than 4.0.

To test the relationship between the rates of ammonium disappearance and nitrate formation with temperature, the natural logarithm of the rates was plotted against the reciprocal of absolute temperature. A straight

line was fitted to give the Arrhenius relationship using the Minitab package by regressing the reciprocal absolute temperature on Ln (rate).

$$Ln\ (K) = Ln\ (A) - B/T$$

The Coefficient of Determination ($R^2$) for the relationship was in the range of 97-99%, indicating that the data fitted well to the Arrhenius relationship. The relationship was found to be improved by omitting the 0 °C data for the Darvel soil and much improved by omitting the 20 °C data for the Darlieth and Freckenham soils. No published Arrhenius coefficients for nitrification in soils were found except those of Addiscott (1983) but he could only show a significant relationship with one out of three soils examined.

The B coefficient of the Arrhenius relationship indicates the temperature sensitivity of the nitrification process. The B coefficient values of ammonium disappearance (-10363 °K to -8521 °K) and nitrate formation (-10246 °K to -8299 °K) were equal for each soil. The B coefficient values of nitrification of this experiment were slightly higher than those Addiscott (1983) reported. The B coefficient values were increased by omitting the values for 20 °C while the B coefficient values were decreased by omitting the 0 °C temperature values.

The mineralisation rates also increased with rise in temperature from 0 to 20 °C in these soils and fitted well with the Arrhenius relationship. The B coefficient values for mineralisation (-8935 °K to -7317 °K) in the present studies agree with the values reported earlier. Tabatabai

and Al-Khafaji found a B coefficient for 12 soils of -8871 °K for nitrogen mineralisation and Addiscott (1983) found -7634 °K for mineralisation.

The effect of two applications of ammonium on five soils was studied by applying ammonium sulphate to soil and measuring ammonium disappearance rates; after complete conversion of ammonium into nitrate a second dose of ammonium sulphate was applied. The calculated rates of ammonium disappearance showed nonsignificant difference between the first and second applications of the ammonium sulphate in four soils having neutral soil pH; however, a significantly lower ammonium disappearance rate was measured in a soil having a pH of 5.8 at the time of the first application and pH 5.2 at the time of the second application of ammonium. This low pH might have decreased the rate.

The climatic data for north western and central England discussed in Chapter 1 indicates that from May to October temperatures reach or exceed 10 °C. Thus applied ammonium fertiliser during this period is very rapidly transformed to nitrate. The results of studies on the effect of temperature show that nitrification rate increased sharply at 10 °C. The ammonium fertiliser applied to arable crops and grass before this period is less subject to nitrification. The arable crops and grass grown can utilise the ammoniacal form of nitrogen fertilisers. Nitrate leaching in the north western area is expected from September to the end of May due to soil moisture content exceeding the field capacity of soils during this period,

while in central areas of England leaching is expected from December to March. The general conclusion of this experiment showed that ammonium transformation even could take place after air drying of soils and at temperatures down to 0 °C. The nitrification of ammonium fertilisers could therefore occur slowly over the winter period.

The minimum mean daily temperature of Pakistan is above 10 °C. The results of this study are only useful for the winter climate of Pakistan because the study was unable to examine the effect of temperatures above 20 °C due to lack of time. The result of the effects of temperature on nitrification rate of ammonium applied fertilisers indicates that the temperature of Pakistan is highly favourable for nitrification activities. The temperature responses of nitrifier populations vary with climatic condition (Malhi and McGill, 1981). The nitrifier population of Pakistani soils could have a significantly different response to the effect of temperature on rates. The soils of the irrigated areas of Pakistan during winter are saturated with water, and nitrates formed can be denitrified or leach down from sandy soils due to heavy rains. Myers (1974) studied the effect of high temperature on nitrification in a tropical soil. Malhi and McGill (1982) reported nitrification rates as effected by temperature up to 40 °C. For studying nitrification in the Pakistani climate the effect of high temperatures ie 25 °C to 50 °C on nitrification and mineralisation need examination, so that the effect of temperature on nitrification rate and mineralisation may be considered for

the summer crops of Pakistan. In addition similar types of studies under field conditions and under fluctuation temperatures will also help in predicting fertiliser timing and application dose.

## 6.3 AMMONIUM CONTAMINATION.

The investigation of the source of ammonium contamination at a former nylon factory site was carried out in four surveys of investigation. In the first investigation the ammonium levels in the surface soils were measured for determining spillage of ammonium on the former nylon site. The surface soil was found not to be contaminated with ammonium. The second investigational survey was conducted by collecting samples with a JCB digger near the previous ammonium storage tanks from the subsoil down to the ground water table to find ammonium levels in the different soil horizons. Difficulties in digging the trial pits at depths greater then 1 metre were seen due to collapsing of the sides of pits because of the loose sandy subsoil. Therefore a small number of samples were obtained. The subsoil samples of the second survey were contaminated with ammonium in one pit out of the three from which samples could be obtained. The investigation was carried forward by obtaining samples by geoprobe boreholes down to 5 metres. These samples were obtained by ICI for an investigation of metal contamination on the site area as part of an environmental inventory. The samples were split to enable ammonium determination as part of this project. Therefore there were limitations on the number of samples

and the sampling points for the ammonium survey. Moreover, in some parts of the site the boreholes were unevenly spread due to the concrete foundation of the factory. As samples were taken for the shallow metal contamination survey, not all of the boreholes were deep enough for the ammonium survey. The geoprobe borehole surveys were conducted in two stages. In the second stage some deeper boreholes were dug.

Most of the samples of the geoprobe borehole surveys were contaminated with ammonium except the samples taken from the south side of the former factory but these samples were taken at shallow depth. The heaviest contamination was found in areas where ammonia was delivered and stored. The highest ammonium level recorded (519 mg $l^{-1}$) was measured in a water sample at 8 metre depth in this area. The level of ammonium contamination in some boreholes appeared to increase with depth below the water table but reached a peak in some boreholes, indicating a layer of high ammonium concentration. The preliminary results of the ground water modelling conducted by ICI, show a flow towards the north east side of the site. Many of the geoprobe boreholes may not have been deep enough to reveal the full extent of ammonium contamination.

Soil properties; loss of ignition, pH and conductivity were measured in all of the soil samples. The measurements indicate that a generally low level of organic matter is present but in some samples a band of high percentage of organic matter was also measured. Soil pH was in the range of 5.6 to 10.0 but did not show any

relationship with increased ammonium contamination. Similarly soil conductivity did not show a relation with contamination. The data on physical properties was measured as part of a collaboration exercise with ICI on modelling of ground water flow and movement of the dominant ions in the ground water. In addition measurements of ammonium nitrogen isotope ratios were carried out at the Scottish Universities Research and Reactor Centre in a attempt to determine the origin of the ammonium as either synthetic or biological. At the time of writing the results of these exercises were not available.

A Geological survey of the area was conducted by digging six boreholes down to solid rock. These six boreholes show different soil mechanics. However, the upper strata in the area are river and marine alluvial sand down to 10 metres. Below this sand lie harder rocks, in the form of carboniferous sandstone, or boulder clay.

In the three profiles of the second survey cation exchange capacity and clay content were measured. The results indicate very low cation exchange capacities (10.7 $mmol_e\ kg^{-1}$), and very low clay content (1.5%) in the soil of this area. A value for the cation exchange capacity of up to 100 $mmol_e\ kg^{-1}$ might be typical of a natural topsoil of the Dreghorn Soil series forming on this type of parent material. The cation exchange sites were dominated by calcium. In the lower part of one profile high levels of ammonium were measured. The ammonium content of 2.3 $mmol_e\ kg^{-1}$ on exchange sites show that a significant proportion (21 %) of the exchangeable cations were in this form. The

proportion of ammonium held on exchange sites or free in the pore solution shows that approximately one quarter of the ammonium was free in the soil solution. The factory was closed down in 1980, but even after the passing of 14 years most of the ammonium is still present at the place where it was utilised. This suggests that the ammonium will continue to leach down for many years.

It is suggested that the possible sources of ammonium contamination are:-

1. The spillage of ammonium on the surface.
2. Corrosion of an ammonium underground storage tank.
3. Contaminated mine drainage

If it is considered that the contamination is due to the corrosion of a tank, then recent corrosion could account for the presence of high levels of ammonium in ground water. The corrosion of an abandoned underground storage tank is discounted as there are no records of underground storage tanks and pipes at the site. The third reason for ammonium contamination by mine drainage is not likely due to the very high levels of ammonium measured and since the contamination is localised. Despite the finding of high levels of ammonium in solution at depth below the watertable after the passage of 14 years, the most likely reason for the contamination is surface spillage during the factory operation period.

In order to determine the full extent of the ammonium contamination it would be necessary to conduct a systematic survey of ammonium contamination by collecting a large number of samples and digging boreholes down to at least 10

metres depth where the hard carboniferous sandstone and boulder clay strata start.

The area may need to be cleaned from ammonium contamination. The contaminated water could be pumped out from the ground through a borehole or a number of boreholes. The pumped water containing ammonium and possibly other contaminants could then be treated by extraction of ammonium and other contaminants. The extraction of ammonium could possibly be done either by distillation or chemically through an ion exchange method. The extraction of ammonium from this water would possibly be expensive.

An alternative method of removal of ammonium from the contaminated pumped water is application to plants. Due to the high ammonium contamination, the high pH and the possible presence of other contaminants such as sulphate and iron, it would be necessary to dilute and neutralise before applying to plants. It is likely to be unsuitable for applying to agricultural crops because of the possible presence of other contaminants. The application of diluted but not chemically treated ground water could only be used for sacrifice areas for growing a crop such as grass. The grass grown by this contaminated groundwater should be tested if it was fed to farm animals, otherwise it should be disposed of.

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