A STUDY OF GROUNDWATER CONTAMINATION AND BIOREMEDIATION TREATMENT USING NATURAL SOIL AND VEGETATION

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THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
SEPTEMBER, 1999.

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IN THE NAME OF ALLAH
"THE MOST GRACIOUS, THE MOST MERCIFUL"

"He Who taught (the use of) the pen,"
"Taught man that which he knew not."

ACKNOWLEDGEMENTS

With sincere words of thanks to the Most Compassionate, the Most Gracious and the Most Merciful, ALLAH Who blessed me, helped me and gave me a light in the road of knowledge.

I wish to express the deepest appreciation and gratitude to my supervisor, Dr. T. H. Flowers for his constant support, continual help and advice throughout the work covered in this thesis. Without his careful attention and encouragement throughout my Ph.D. this thesis could never has been completed.

I am extremely grateful to Dr. H. J. Duncan, Dr. I. D. Pulford, and Dr. Jarvis for their sincere help and advice during the course of this work. Their encouragement and support have been of great value to me and appreciated.

I wish to thank all colleagues and friends both past and present who have been of assistance in my ways.

I am extremely grateful to the Ministry of Higher Education in Egypt and to all the staff of the Egyptian Educational and Cultural Bureau, Embassy of Arab Republic of Egypt, in London, especially Prof. Mohammed Abd El-Hamid El-Sharkawy for their encouragement, sincere help and financial support of my study and living.

I am extremely grateful to all the staff members of the Soil Science Department, Faculty of Agriculture, Minia University, Egypt, for their sincere help and advice. Their encouragement and support have been of great value to me and appreciated.

I wish to thank Mr. Robert Craig for his help in collecting my groundwater and soil samples from the Ardeer site, Mr. Fulton Murdoch for his help with analysis by Inductively Coupled Plasma (ICP) Spectroscopy, and Mrs. Janet Harris, Environmental Scientific Officer, for the information relating to the Ardeer site she supplied.

I am extremely grateful to my father and the soul of my mother for their sincere help, support and encouragement throughout my life.

I am extremely grateful to my wife for her constant patience, support and encouragement.

Finally, I wish to express my sincere thanks to my 8 months old daughter Omnia Hassan Ali Hassan for keeping quiet.

CONTENTS

SUMMARY

The Ardeer site of Nobel Enterprises has been used for the manufacture of a range of chemicals and explosives such as Nitro-cellulose, Nitro-glycerine, Dyestuffs and Nylon, and their associated acids, predominately Sulphuric and Nitric acids. A small part of the site was also set aside as a licensed landfill facility. As a result of some of these activities localised contamination has occurred. The particular interest of this study is the nitrogen contamination of the groundwater associated with the areas of the site where the groundwater has been contaminated by ammonium and nitrate. The focus of the current study is to investigate the distribution and concentration of ammonium-N, nitrate-N and other contaminants such as chloride, sodium, potassium, calcium, magnesium, and iron in the groundwater at the Ardeer site, to identify the sources of nitrogen contamination, to evaluate the groundwater quality parameters, and to assess the effects of groundwater composition on nitrification in soil. In addition, the intention was to look at the possibility of bioremediation treatment of the groundwater. The bioremediation treatment being considered is by pumping well water to the surface, irrigating the vegetation, and harvesting the vegetation [as vegetation such as perennial ryegrass (*Lolium perenne* L.) uses ammonium and nitrate as a fertiliser before it leaches down in soil profile]. This bioremediation treatment could be applied to other circumstances in which groundwater is contaminated.

This thesis is concerned with the following studies :

1- Ammonium analysis in soil and water including the determination of low levels of ammonium (< 0.1 mg N / l) in groundwater and the colorimetric analysis of highly coloured groundwater samples.

2- An investigation of groundwater quality and soils at an contaminated industrial site.

3- Bioremediation treatment of the ammonium and nitrate contaminated groundwater using natural soil and vegetation and using soil incubation and pot experiments.

Regarding the optimisation of the method of ammonium-N determination on a Technicon Autoanalyzer II system, the results of the evaluation of the precision of the optimised method were very reproducible as shown by the low RSD % and were

considered acceptable since they were less than 0.5 %. Comparison of the optimised method with those of other authors suggested that it has very good RSD % values and was suitable for the analysis of ammonium-N in soil and water.

In addition, analytical studies were accomplished to develop a reliable Technicon Autoanalyzer method to determine low levels of ammonium in groundwater (< 0.1 mg /l). The results of the evaluation of the developed method were very reproducible as shown by the low RSD % and were considered acceptable since they were less than 0.5 %. Comparison of this low level ammonium - N method with the optimised method showed that both have very good RSD % values and were suitable for analysis of low levels of NH_4-N in groundwater.

Further analytical studies were undertaken to develop a method for decolorisation which can cope with highly coloured groundwater samples prior to colorimetric analysis. The investigation using charcoal G60 and polyclar SB100 for the decolorisation of water samples suggested that the charcoal G60 was not suitable to be used for decolorising the water samples since the cleaned charcoal became contaminated with ammonium and adsorbed nitrate from the solution. The polyclar SB100 was also not suitable to be used for decolorising the water samples because the cleaned polyclar SB100 adsorbed NO_3-N from the mixed standard solution. In addition, K_2SO_4 solution prevented the ammonium adsorption by the polyclar but not the adsorption of nitrate.

The need to decolorize the groundwater samples required the development of a dialysis system which could be included with the Technicon Autoanalyzer II system. From the investigation of the inclusion of the liquid phase dialysis with the Technicon Autoanalyzer II, a method for the determination of nitrate and nitrite in the groundwater samples was developed. The evaluation of the linearity of the developed method revealed that the addition of the buffer borate and reducing reagent solutions on the acceptor side of the dialyser and the addition of the buffer borate solution on the acceptor side of the dialyser gave a linear calibration graph, but the addition of the buffer borate and reducing reagent solutions on the acceptor side of the dialyser was more convenient.

The evaluation of the linearity of the method used for the determination of chloride in the groundwater samples by including the liquid phase dialysis with the Technicon Autoanalyzer II demonstrated that the method had a linear calibration graph. Very slight curvature was a characteristic of this method (A NON, 1982).

The groundwater quality surveys at the Ardeer site showed that the majority of the wells had pH in the range from 6.1 to 8.3 but some wells were acidic (pH below 4.3). Ammonium contamination was quite localised at the site. The nitrate contamination was also very closely localised but ammonium and nitrate were not necessarily found together. Chloride, sodium, potassium, calcium, magnesium and iron contamination was widespread at the site. High electrical conductivity was widespread at the site. Well No. SW 15 was the most badly contaminated well on the site.

The soil survey at the Ardeer site demonstrated that in the IOP (Intermediate Oxidation Plant), H-Acid, Safety Fuse and Nylon areas; the pH was slightly acidic (the pH was slightly higher in the case of the Nylon area). The electrical conductivity was low. The organic matter content was low (the organic matter content was high in the case of the H-Acid area). Ammonium and nitrate levels were very low. Phosphorus and potassium levels were low (phosphorus and potassium levels in the H-Acid area were slightly higher than those of the IOP area). The levels of toxic metals were low in the soils.

It was concluded that the heavy metals were not expected to cause problems with growth of vegetation or microbial nutrients turnover. In some cases, the pH was borderline for nitrification, although none of the sites appeared likely to cause problems for vegetation growth. Overall, the variations in vegetation cover appeared to be due to differences in macronutrient availability rather than any toxicity. Many soils were very sandy and prone to leaching and low in organic matter content and available nitrogen.

The preliminary experiment of nitrification was carried out to evaluate the four soil samples of the Ardeer site plus two control soils (garden natural soils) for their nitrification rate. The soils were treated with 100 mg NH_4-N /kg soil, incubated at -0.5 bar moisture potential and 20 °C. From this preliminary experiment, it was concluded that the Safety Fuse and Nylon soils had extremely low nitrification rates and were not suitable for further studies. The IOP and H-Acid soils were suitable for subsequent work together with two garden natural soils (Darvel and Dreghorn) which were used for comparison.

The nitrification experiment was carried out using two soil samples from the Ardeer site and two natural garden soils. The soils were treated with three well waters to supply 100 mg NH_4-N /kg soil, incubated at -0.5 bar moisture potential and 20 °C. It

was found that well water No. SW 11 showed no inhibition in all four soils. Well water No. SW 15 showed significant inhibition in the IOP, Dreghorn and H-Acid soils but not in the Darvel soil. Well water No. TW 8 significantly inhibited all the soils to the extent that nitrification was barely measurable in the Dreghorn, IOP and H-Acid soils. The effect of inhibition was less in the Darvel soil and was greatest in the sandy textured soils (Dreghorn and IOP soils). The inhibition in nitrification caused by well water No. TW 8 could be explained by the presence of boron.

The pot experiment for the bioremediation of the ammonium and nitrate contaminated groundwater was carried out using four soil samples and three well water samples. Each soil sample was treated with five treatments. Nutrients (nitrogen, phosphorus, potassium and magnesium) were added to the soil as a nutrient solution and the soils were cultivated with perennial ryegrass seed [*Lolium perenne* L.] under controlled conditions in the greenhouse. It was concluded from this pot experiment that well water No. TW 8 had a clear negative effect on the growth of grass as the yield of this treatment was the lowest in all four soils in the first period and there was no growth in the second period. Toxicity symptoms appeared on the grass of the pots treated with this well water. The toxicity effects were severe in the IOP, Safety Fuse and Nylon soils but were less so in the H-Acid soil. Among the well water treatments, well water No. SW 15 (single treatment) was superior in producing dry yield in all four soils.

The grass treated with well water No. SW 15 (split treatment) and the control treatment (single treatment) was more efficient in nitrogen uptake than that treated with well water No. SW 15 (single treatment) in all four soils. The total nitrogen yield in the shoots of grass grown in the H-Acid soil was higher than that of grass grown in the other three soils, however, the total nitrogen yield in the shoots of grass grown in the Safety Fuse soil was lower than that of grass grown in the other three soils.

Ammonium and nitrate were the dominant ions remaining in the soil at the end of the pot experiment, but ammonium level was higher than the nitrate and nitrite levels in all four soils. The residual nitrogen in the H-Acid soil was higher than that in the other three soils. There was very little nitrogen remaining in all four soils as inorganic nitrogen only 0.2 - 2.46 % of nitrogen applied.

These findings of the pot experiment suggest the possibility of applying the bioremediation treatment of the ammonium and nitrate contaminated water in the field. A field study should be undertaken to evaluate the efficiency of this bioremediation treatment. This field study would require a suitable uniform area to lay out the plots, preferably close to the source of water to be used. In addition, it is necessary to carry out a hydrological survey to determine the following aspects :

1- The size of groundwater reservoir.
2- The rate of removal of the water.
3- The time scale of the water application.

The climatic conditions such as rainfall, potential evapotranspiration and temperature should be taken into consideration when carrying out the bioremediation treatment in the field as these climatic conditions affect the water requirements and the growth of grass. There are three options to apply the contaminated groundwater as follows :

1- To apply the contaminated groundwater at low or high volume depending on its level of nitrogen.
2- To blend well water with high level of nitrogen with well water with low level of nitrogen to achieve a realistic irrigation rate at a suitable nitrogen level.
3- To overirrigate in expectation that ammonium would be retained in the soil.

The ryegrass used in this bioremediation treatment can be disposed of by the incineration and landfilling the ash or landfilling the grass.

CHAPTER 1

GENERAL INTRODUCTION

Water is an essential component for humans, plants and animals. Not only the quantity of water but also the quality of water is important. As the population continues to increase all over the world, it is necessary to find additional sources of water such as groundwater and drainage water. Groundwater has become an important source of fresh water in many countries. However, human and industrial activities have had an adverse impact on the quality of groundwater, consequently the quality of groundwater has deteriorated in the last three decades. The occurrence of contamination in groundwater is a warning that public health, soil properties and plant growth are in serious and great danger. Therefore, there is a need to treat contaminated groundwater to avoid this threat. Groundwater contamination in general, and ammonium and nitrate contamination in particular, have generated much interest. One possible method of cleaning ammonium and nitrate contaminated groundwater is by bioremediation. This process could be carried out by irrigating ryegrass with ammonium and nitrate contaminated groundwater, as the ryegrass uses the ammonium and nitrate as fertiliser before the nitrate leaches down in the soil profile. In order to carry out this bioremediation process it is important to understand the nitrogen cycle in general and the nitrification process in particular. The slow ability of soil to nitrify ammonium in the irrigation water allows ryegrass to use it as fertiliser before it leaches down in soil. The slower the rate of nitrification in the soil the greater the opportunity for the grass to take up the nitrogen before it is converted to nitrate and lost from the soil profile by leaching.

1.1. NITROGEN CYCLE

Nitrogen is an important major element for plants. The Nitrogen cycle includes the nitrification, denitrification, mineralization and immobilisation processes.

Green and Shelef (1994) indicated that nitrogen is an essential element for all organisms. The main source of nitrogen to animals is provided by plants. In addition,

the natural sources of nitrogen for the plants are mainly animal excreta, animal and plant remains, and in some special cases also atmospheric nitrogen. A direct, natural result of the nitrogen cycle is the contamination of water by inorganic nitrogen compounds, and the nitrate ion, due to its chemical stability, is the major contaminant.

Nitrogen added to the soil as inorganic fertilisers provides nitrate (NO_3-N), ammonium (NH_4-N) and simple amides (- NH_2). In addition, animal manures contain both ammonium and organic forms. The loss of nitrogen occurs by leaching through the soil to the drains or aquifer and also in gaseous form to the atmosphere. Gaseous nitrogen (N_2) is fixed from the atmosphere. The main chemical forms of nitrogen in the soil-crop system and the directions in which changes may occur are shown in Figure 1.1. Nitrogen changes from one form to another as the soil organic constituents change. Nearly all these transformations of nitrogen are carried out by soil micro-organisms (Archer, 1988).

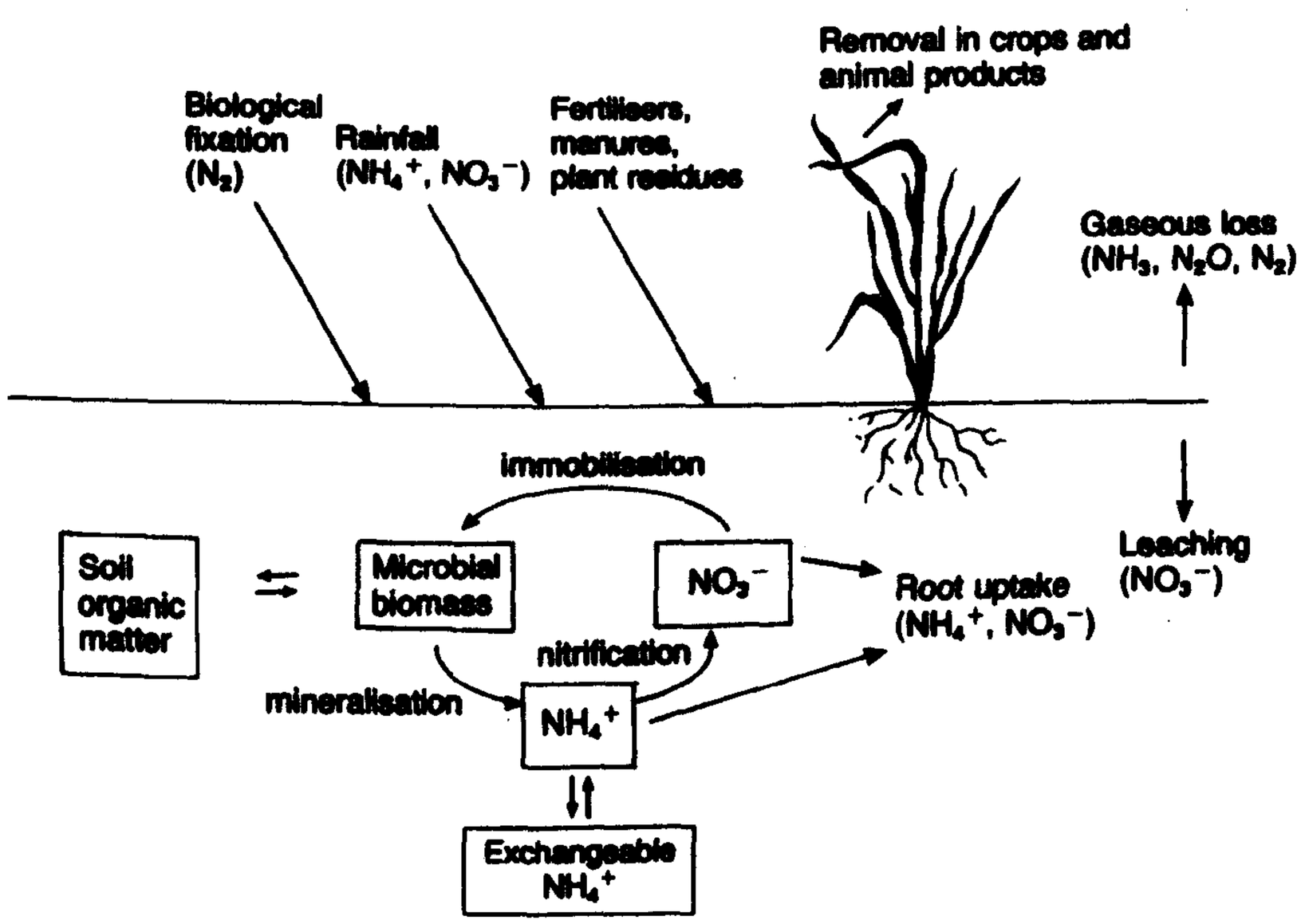


Figure 1.1. : The nitrogen cycle according to Archer (1988).

Inorganic nitrogen in soils is ephemeral, growing crops take it up quickly but that not used by plants is likely to be leached or denitrified. The large and long-lasting

reserves of N in soils are all combined with organic matter. A part of the organic matter in most soils is very ancient, however, part is derived from recently added crop residues or organic manures. A small part of the old organic matter is decomposed to release a few kg of N each year. The turnover of the more recent organic matter is much quicker and soils containing residues of leguminous crops, grassland, or organic manures may release 100 kg N /ha /year, or even more (Cooke, 1982).

1.1.1. PLANT UPTAKE

Nitrogen is the nutrient required in the greatest quantity by most crops. It is also one of the most complex in behaviour; occurring in soil, air and water; in inorganic and organic forms. Therefore, it provides the most difficult problem in making fertiliser recommendations for crops (Archer, 1988).

Ammonium and nitrate ions can be taken up by most crop plants through the root system but nitrate is the most uptake at normal soil pH levels for crop production. This is due to the rapid conversion of ammonium to nitrate in the soil following application of any ammonium fertilisers. Within the plant, the transformation of nitrogen occurs by the reduction of nitrate by the enzymes nitrate reductase and nitrite reductase to ammonium. Further enzymes then convert ammonium to simple soluble organic molecules, amino acids, amides and amines. This stage is not reversible in crop plants and is followed by conversion to proteins, the dominant nitrogen fraction in green plant material, and nucleic acids. As the nitrogen is translocated from the roots to the youngest parts of the plant, these nitrogen transformations occur at several places within the plant. Nitrogen is very mobile in the plant. Therefore, any shortage of nitrogen in young tissue is generally met by mobilising compounds from the older leaves and this results in a loss of chlorophyll showing as yellowing or chlorosis (Archer, 1988).

As grassland farming becomes more intensive and more nitrogen is used, it becomes increasingly more important to maintain a balance between all the three major nutrients; N, P and K. Failure to do this can result in a disappointing response to nitrogen and poor grass growth overall. Potash is the most important mineral to keep in balance with nitrogen. Nitrogen stimulates growth and produces leafy, high-protein grass. Moreover, it is quick-acting, being readily taken up and utilised. Grass needs nitrogen throughout the growing season.

If phosphate and potash levels are adequate, nitrogen can be used to manipulate

both the rate and the amount of grass growth as the rate of grass can be speeded up dramatically with nitrogen. Grass responds linearly to increasing amounts of nitrogen up to about 350 kg per hectare. However; from then on the response levels off until, at about 500 kg per hectare; there is little or no extra growth. Each kg of nitrogen applied up to the 350 kg per hectare mark is producing about 25 kg of dry matter in an all-ryegrass sward.

If applied too early, the nitrogen is lost; and if applied too late, the grass growth is lost. Therefore, at the beginning of the season the right time to apply nitrogen is as soon as the process of grass growth starts. The problem at the end of the season is when to stop applying nitrogen. It is too late by mid-September in most areas. In addition, an Autumn application is too late when it continues to promote grass growth after the stock have been housed. Late nitrogen applications increase protein content which in turn reduces the sugar content. Sugar acts as antifreeze and a reduced sugar content makes winter kill more likely (Craven et al., 1981).

Plants take up both ammonium and nitrate ions. Except in very acid soils ammonium-N is quickly converted to nitrate by microbial action; where this conversion is slow due to extreme acidity, plants adapted to the conditions may take up much ammonium (Cooke, 1982).

1.1.2. MINERALIZATION / IMMOBILISATION

Mineralization is the process in which soil organic matter can be transformed first to amino-N and then to ammonium-N by a wide range of heterotrophic micro-organisms, using carbon as their energy source. As this process is carried out by many soil bacteria and fungi, the rate of change is limited by the environment rather than by a lack of the right micro-organisms.

The use of the organic materials by micro-organisms may result in release of ammonium-N or all the nitrogen mineralized may be incorporated into the organisms themselves as they multiply in the soil. In the case of a very low nitrogen content of the particular organic matter source, the organisms will need extra nitrogen to grow, therefore, inorganic nitrogen already present in the soil will be used up by the soil micro-organisms. This process is called nitrogen immobilisation (Archer, 1988).

Clein and Schimel (1995) indicated that the shifts in relative C and N limitation to the soil microbial communities was responsible for the changes in N dynamics in

reciprocal transplants. When neither element limited microbial communities, gross N turnover was greatest but net N availability increased as the microbes became more C limited. This had three major implications for understanding N cycling in this system. First, gross mineralization and immobilisation were intimately linked. While they shift somewhat relative to each other, changes in one lead to changes in the other as well; this was probably due to changes in biomass N turnover. Second, N availability to nitrifiers (and, therefore, probably to roots as well) was controlled by the balance between gross mineralization and immobilisation, rather than by the rate of NH_4-N production or NH_4-N concentration. Third, though balsam poplar compounds have been cited as allelopathic nitrification inhibitors, the changes in nitrification when poplar invades an alder site appear to be controlled by changes in N turnover and availability resulting from the changing balance of C and N inputs into the soils.

1.1.3. DENITRIFICATION

Denitrification is the anaerobic process in which microbes convert NO_3-N to N gases in the absence of oxygen (Hanson et al., 1994).

Wild (1988) indicated that one of the most important causes of soil and fertiliser nitrogen loss as gaseous products is microbial denitrification, that is, the reduction of nitrate to nitrous oxide (N_2O) and nitrogen gas (N_2). The most common bacteria responsible for the reduction of gaseous forms of nitrogen are the heterotrophs such as *Pseudomonas* and *Alcaligenes* although certain autotrophs such as *Thiobacillus denitrificans* can reduce nitrate in the course of oxidising sulphur compounds.

Losses from arable soils are higher than from grassland soils since the latter tend to maintain lower nitrate levels. Nitrate will be lost from soil most rapidly when it is warm, wet and well supplied with easily decomposable organic matter. In addition, organic additions by providing a substrate for bacterial growth, are liable to enhance denitrification providing that nitrate concentrations are high. Nitrate loss can double with a temperature increase of 10 °C over the range from 10 °C to 35 °C, while in the range from 0 °C to 5 °C denitrification is much reduced but still measurable and the proportion of nitrous oxide to dinitrogen gas increases. There is a positive relation between denitrification and pH, the process taking place more readily in neutral and calcareous soils than in acid situations with a peak in the region pH 7 to pH 8.

1.1.4. AMMONIA VOLATILISATION

Wild (1988) indicated that ammonia is lost under high pH condition. At pH 7.0 about 1.0 per cent of the ammonia/ammonium in solution is present as NH_3, but the percentage increases rapidly as the pH is increased. Under field conditions, where the soil surface is open to the atmosphere, volatilisation varies with the rate of transport of NH_3 away from the surface, which is determined mainly by wind speed, and with evaporation of water from the surface. Loss occurs from soils, or microsites within soil, which are at pH 7.0 or above, especially if the ammonium is present in a drying soil surface. It can be severe following applications of urea fertiliser to the soil surface because of the rise of pH when the urea is hydrolysed to NH_4^+ and HCO_3^- (and CO_3^{2-}). The cation exchange capacity of the soil has an effect because the greater the proportion of the ammonium ions held on exchange sites the lower the concentration in solution.

1.1.5. NITROGEN FIXATION

Fixation of atmospheric nitrogen as ammonia can be carried out by a number of micro-organisms. There are two types of nitrogen fixation; one is carried out by free-living micro-organisms in the soil and the other by micro-organisms living in symbiosis with plants. The biochemical mechanism for each is probably the same, both involving the enzyme nitrogenase. Agriculturally, symbiotic fixation is far more important. Legumes and a few other species have the ability to fix atmospheric nitrogen. The legumes carry out the fixation in symbiosis with the soil bacteria, *Rhizobium*, which takes place in nodules located on the plant roots. Nodule formation follows infection with the appropriate strain of *Rhizobium*. The effectiveness of the symbiosis depends on environmental conditions, fixation is inhibited below pH 6.0 and by high nitrogen fertiliser use. Well-aerated soil conditions are needed for good nodulation (Archer, 1988).

1.1.6. NITRIFICATION

The conversion of ammonium-N first to nitrite-N and then to nitrate-N mediated by specific soil bacteria, *Nitrosomonas* and *Nitrobacter* is called nitrification (Archer, 1988).

The conversion of ammonium ions to nitrate is essential for the growth of some plants, as they are able to absorb nitrate but not ammonia or ammonium. Unfortunately, nitrate is very soluble in water and easily leached from soils, and denitrification is also important in some soils, therefore a build-up of a nitrate reserve in the soil is not possible (O'Neill, 1993).

1.1.6.1. FACTORS AFFECTING NITRIFICATION AND MINERALIZATION

As all field soils contain *Nitrosomonas* and *Nitrobacter* bacteria, the change from ammonium to nitrate will occur as long as pH, temperature and moisture levels are satisfactory. As the second stage of the process is more rapid than the first, nitrite does not accumulate. The factors that affect on the rate of the nitrification are soil water content, oxygen supply, organic matter, pH and temperature. The rates of nitrification are much reduced below pH 5.0. Nitrification does not occur in the absence of oxygen. As with most soil biological processes, the rates of activity decline rapidly if the soil is waterlogged or too dry. Temperature has a marked effect on rates of nitrification, over the soil temperature range 5 - 30 °C, the process is rapid; however, nitrification occur much more slowly below 5 °C. (Archer, 1988).

The rates of mineralization in the soil depend on several factors such as soil water content, oxygen supply, organic matter, pH and temperature. Soil water content is important because as the soil dries, biological activity declines rapidly. Oxygen supply must also be adequate as soil organisms require oxygen to function. However, if soil oxygen supply is low due to waterlogging, breakdown may still occur by bacteria which can function in the absence of oxygen. The rates of breakdown of organic matter are slower under acid conditions than when the pH is 5.0 or above and this is mainly due to the restricted range of soil flora and fauna that exist under acid conditions. Temperature is the other major factor determining the rates of breakdown, over the range 10 - 30 °C, an increase of 10 °C increases the rate of microbial activity by two or three times (Archer, 1988).

Ishaque and Cornfield (1972) conducted a study on the effects of increasing pH, by addition of varying levels of calcium carbonate on N-mineralization and nitrification during aerobic incubation (30 °C for 12 weeks) of two "tea" soils (original pH 4.1 and 4.2) from East Pakistan. They reported that the accumulation of mineral-N (NH_3 plus

NO_3-N) increased with the pH in both soils. In the low-flat soil, maximum nitrate accumulation occurred at pH 5.0, however, at the higher pH levels mineral-N accumulated mainly as ammonia-N. In the high-flat soil, nitrate accumulation increased considerably with the pH; but mineralized-N was accounted for largely as ammonia at pH 5.0 or less, and almost entirely as nitrate at higher pH levels.

Wild (1988) pointed out that the presence of a nitrifying population and the ammonium substrate are the main requirements for nitrification to take place in a field soil. There are several factors affect on nitrification such as temperature, soil moisture and pH. Nitrifying bacteria have a high optimum temperature for activity and reach a maximum at about 25 to 30 °C. Soil moisture has a considerable influence on nitrification both by itself and through its effect on aeration. For example; at low moisture contents microbial activity is depressed and mineralization of organic nitrogen will be slow, hence limiting the amount of ammonium available. Nitrification does not take place readily in very acid soils with the possible exception of limited heterotrophic nitrification.

Grundmann et al. (1995) showed that soil temperature and soil moisture probably have the greatest influence on nitrification due to their importance in soil aeration. The conditions of relatively high water contents may have particular importance as substrate diffusion becomes less limiting and O_2 diffusion becomes more limiting for aerobic microbial activity, consequently leading to denitrification. Temperature may interact with the water content and influence the diffusion of O_2 and CO_2 in soil water, and consequently O_2 distribution, depending on soil structure.

Fdz-Polanco et al. (1994) emphasised that the activity and population of the nitrifying bacteria depend on specific free ammonia concentration (ratio NH_3 /biomass), that is a function of temperature, pH, ammonium concentration and nitrifying biomass concentration. Therefore, temperature is a key parameter in the nitrification process producing two opposite effects : bacteria activation and free ammonia inhibition.

1.1.6.2. INHIBITION OF NITRIFICATION

Inhibition of nitrification occurs by various toxic materials present in the soil. One of these toxic materials is ammonium. As ammonia is toxic to both groups of organisms, high concentrations of ammonia fertiliser will reduce the rate of its

subsequent conversion to nitrate. Partial soil sterilants such as methyl bromide or dazomet which are commonly added to soils cause an initial decrease in nitrification, often followed by an increase later in the season as biological activity resumes again. Moreover, the specific chemical inhibitors such as nitrapyrin (N-serve) and dicyandiamide (Didin, DCD) reduce nitrification. Both materials inhibit nitrification when added to the soil. Furthermore, these materials reduce the rate of ammonium conversion to nitrate at soil temperature above about 6 °C, when added to the fertiliser. The length of time that the inhibitor delays nitrification depends mainly on temperature, therefore, the breakdown of these chemicals is much quicker in summer than in autumn or spring (Archer, 1988).

Bohm (1994) developed a biotest to investigate wastewaters for the presence of nitrification-inhibiting substances. He summarised that inhibition could be found even when the wastewater was diluted considerably. The use of Tannary sewage may result particularly in severe problems in biological wastewater treatment, as the degree of inhibition of this wastewater has been observed to be similar to that of a solution of 2.0 mg /l allylthiourea. With the determination of the nitrification rate by analysing ammonia removal, wastewaters with high concentrations of organic fixed nitrogen cause problems as a result of the ammonia production during the test phase.

Fdz-Polanco et al. (1996) showed that the free ammonia inhibition effect highly depends on the values of pH, temperature and ammonium concentration. For instance, in situations of free ammonia inhibition, the combined effect of temperature, pH and ammonium concentration bring about different nitrite accumulations for the same specific free ammonia concentration, which may explain the differences found in the literature for the thresholds of free ammonia inhibition established by many authors. Therefore, any inhibition situation should be defined by the values of free ammonia, ammonium and biomass concentrations, pH and temperature. In conditions of no free ammonia inhibition and low values of temperature and pH, high ammonium concentrations bring about a higher relative activity of ammonia oxidiser micro-organisms and then, a nitrite accumulation may happen in the system.

1.2. GROUNDWATER QUALITY

Water quality has the most concern due to its impact on humans, animals, soil and plant. Quality of water depends on its chemical and microbiological composition. The suitability of water for irrigation, drinking, industrial activities, and other purposes depends on its quality. The chemical parameters that determine the water quality are total soluble salts; the proportion of sodium to calcium and magnesium; nitrogen (NH_4-N and NO_3-N); bicarbonate; pH; and toxic ions such as sodium, chloride, boron and heavy metals. Water quality deteriorates as the water becomes contaminated. Poor quality water has a serious impact on the chemical and physical properties of soil and plant growth.

Water has the major importance to the survival of life on the Earth. Water covers about 70 % of the Earth's surface and its properties and vapour control the climatic conditions that make life possible on Earth. In addition, water's solvent properties control the chemical weathering of rocks, the transfer of nutrients to plants and the transfer of chemicals inside organisms.

Water within the surface zone of the Earth is distributed as follows : 97 % is in the ocean, about 2 % is in ice caps and glaciers, which cover 10 % of the present land surface, and only 0.6 % is fresh water of direct use to humans. The water cycle (Figure 1.2.) is driven by the absorption of solar energy which causes evaporation of water from the oceans and land, however, a small proportion generates the winds, waves and currents that aid the circulation in both the atmosphere and water masses. Of all the evaporated water, 86 % comes from the oceans, but only 78 % of the rain and snow that falls comes down on the oceans. The evaporation of water which requires the absorption of energy results in reduction of the temperature at the air /water interface (O'Neill, 1993).

Over 90 % of the fresh water resources is groundwater, it is therefore, an important reserve of good quality water and naturally contributes to river flow, lakes, and soil moisture, particularly when rainfall is low. When rainfall is low or absent, groundwater can be considered as a natural water resource due to its buffer capacity (Mandl et al., 1994).

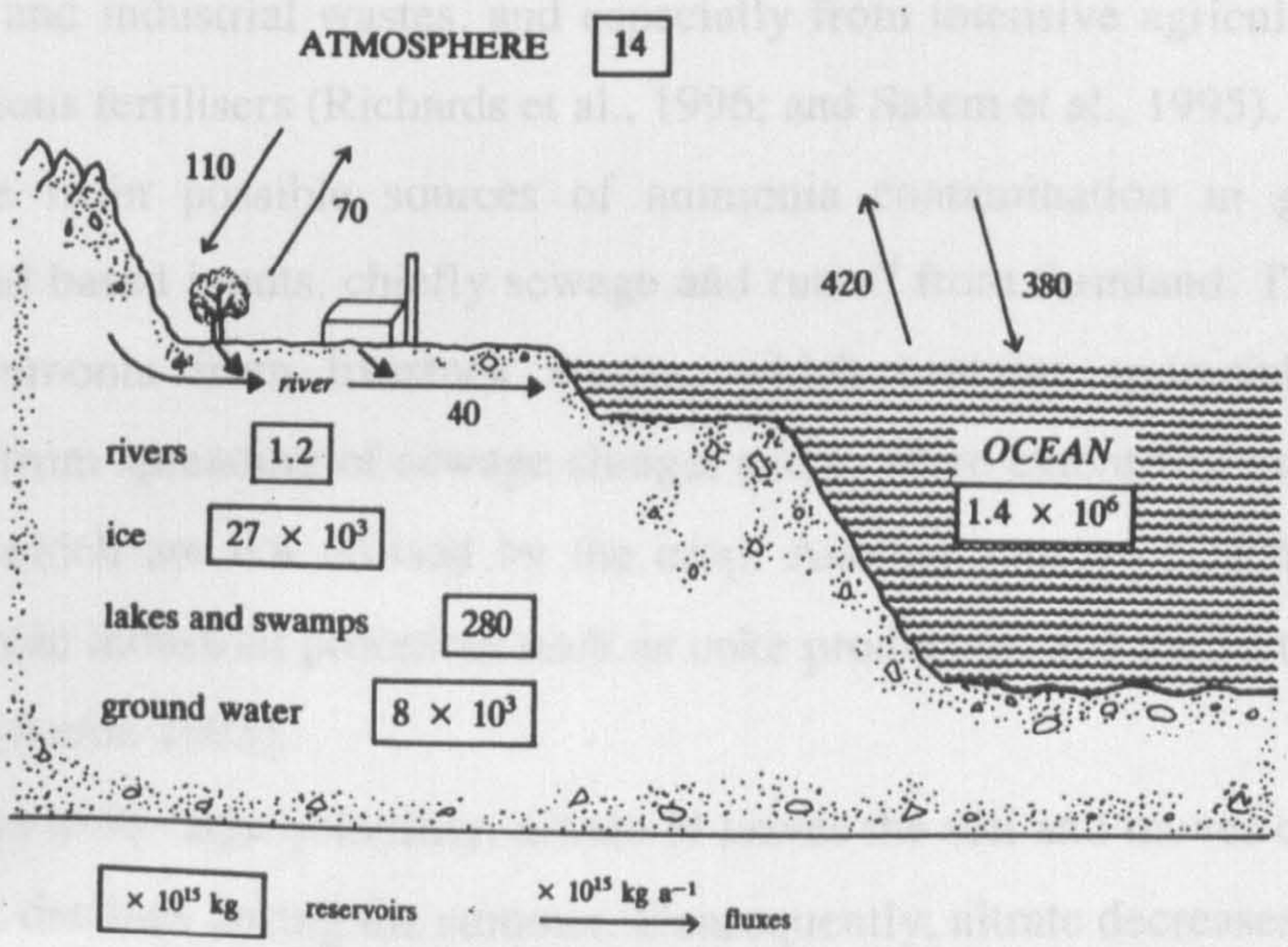


Figure 1.2. : The water cycle, in simplified form, according to O'Neill (1993).

1.2.1. SOURCES OF GROUNDWATER CONTAMINATION

Kent and Spycher (1994) showed that the chemical character of groundwater is acquired primarily through chemical reactions between the water and the mineral assemblages that contact it. Therefore, it depends on (1) the main mineralogical composition and lithological texture of the subsurface, (2) the water solubility of the rock-forming chemical phases, (3) the prevailing water temperature and pressure, (4) the amount of time the water remains in contact with given rocks or sediments, and (5) other parameters such as pH, dissolved oxygen, presence of organic matter, complexing agents, etc. Because of its acidity, rain water that percolates to the water table has a tendency to dissolve minerals from rocks and sediments. With slow recharge , the water that infiltrates the unsaturated zone has time to react with minerals and also to evaporate partly, therefore, its salinity may increase significantly. With rapid infiltration, the recharging water may contain only low concentrations of dissolved minerals.

Nitrate may reach groundwater via the soil nitrogen cycle or directly through cracks and fissures in the soil profile. A major source is from the turnover of soil

organic matter, often stimulated by cultivation of the soil. Other sources include N_2 fixed by leguminous plants, livestock manure, feedlots, septic tanks, land application of municipal and industrial wastes, and especially from intensive agricultural application of nitrogenous fertilisers (Richards et al., 1996; and Salem et al., 1995).

The main possible sources of ammonia contamination in groundwater are usually land based inputs, chiefly sewage and runoff from farmland. The other sources include ammonia from livestock wastes, which contains appreciable amounts of ammonia, from spreading of sewage sludge, and to some extent from the proportion of fertilisers which are 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 (Amin, 1995).

Due to its large solubility, nitrate-N leaves the soil and moves downward as the water table declines during the summer. Consequently, nitrate decreases in the soil zone and increases in groundwater (Blevins et al., 1996).

Nitrate concentrations decrease with groundwater depths and they are also highest at the top of the water table and decrease with depth. Therefore, in the older irrigated areas, which generally have shallow water tables (less than 30 m), nitrate contamination is often prevalent due to leaching through the vadose zone (Abu Zeid and Biswas, 1990).

Abu Zeid and Biswas (1990) showed that the contamination of water, particularly groundwater due to the substantial amount currently used for domestic purposes, by pesticides and nitrates has become an important concern primarily in Europe and North America. As agricultural activities have numerous impacts on water quality, similarly water quality considerations have important implications for agricultural activities. The major impacts of agricultural activities on water quality include the following :

1- Alterations in sediment load due to changes in land use practices.

2- Changes in salinity due to agricultural activities.

3- Water quality deterioration due to anthropogenic chemicals like nitrates and pesticides.

4- Possible eutrophication of water bodies due to leaching of fertilisers.

5- Quality degradation of water due to agroprocessing industries.

The magnitudes of these impacts can be reduced only by better management practices which invariably include some efficient methods for controlling the source of

the potential pollutants, e.g. better land use practices to ensure less generation of sediments, more efficient use of fertilisers and insecticides which will minimise water contamination, and higher levels of treatment of wastewater from agroprocessing industry.

Mandl et al. (1994) reported that the sources of the pollution of groundwater are :

1- Pollution by fertilisers, including livestock manure and pesticides used for agricultural or non-agricultural purposes.
2- Pollution from industrial, urban and mining areas as well as from transport infrastructure.
3- Pollution from old industrial sites and waste tips, sewage sludge disposal and irrigation with polluted water.
4- Indirect pollution of groundwater by atmospheric deposition on soil.
5- Pollution through soils and surface waters due to the discharge of untreated wastewaters or by disposal of industrial or mining waste.
6- Accidental or other pollution from defective fixed industrial installations, storage of dangerous substances, dumps and landfill sites, and individual treatment systems.
7- Direct pollution due to the leakage from one aquifer to another, notably through incorrectly drilled abstraction wells and large underground excavations during civil engineering works.
8- Accidental pollution from transportation activities.

Melloul and Goldenberg (1994) indicated that as the human activity produces harmful materials commonly called "pollutants" or "contaminants", the monitoring of contaminated aquifers is an important part of modern human-environment interaction. The contaminated liquids and leachates result when pollutants are carried by or in water. The introduction of these contaminants into the vadose and the saturated zone of aquifers, results in degradation in the quality of groundwater. The main sources of groundwater contamination are :

1- Sites of disposal of solids and liquid waste materials. The waste may include industrial material, toxic raw materials, etc.
2- Sites of disposal of sewage (including sanitary landfills) and water-treatment plant sludge.

These sources may generate leachate contaminated by decomposed organic matter, inorganic salts, heavy metals, bacteria and viruses.

3- Agricultural areas that produce contaminants containing fertilisers and pesticides.

4- Airborne sources that contribute dangerous components existing in smoke, dust, or aerosols.

5- Sites where spreading of wastewater on land surface is practised. Contaminated surface waters like lakes and rivers.

6- Sea encroachment.

The pollutants of most concern in the monitoring of contaminated aquifers are the soluble substances including heavy metals (e.g., lead, cadmium, chromium, mercury, nickel, cobalt, beryllium, vanadium, zinc and tin), organic compound like pesticides and insecticides, oils and their derivatives, dense non-aqueous-phase substances (DNAPL) (e.g., halogenated hydrocarbons), as well as micro-organisms and their toxins.

Altman and Parizek (1995) showed that the nonpoint-source pollution from agriculture can cause the degradation of groundwater and surface water. One of the method to control the nonpoint pollution problems is changing the farming practices, for example, changing manure management practices, tillage methods or the cropping systems, or lowering fertilising loads. In addition, the natural processes such as uptake by vegetation, denitrification and microbial immobilisation that might control nonpoint-source pollution from farming have the same mechanism by which NO_3-N can be removed from the groundwater under specific conditions.

Lee et al. (1994) showed that heavy metals can be retained by soils by many processes including sorption, complexion and precipitation. Since these mechanisms affect their solubility or mobility into the groundwater, groundwater contamination by metals is heavily dependent on the extent of their sorption by soils. Metal sorption by the soil is highly dependent on the solution pH. Moreover, there are other important factors controlling metal sorption such as organic matter, cation exchange capacity, and the presence of metal oxides. In the groundwater contamination point of view, anions of more concern than cations because anions are normally not well retained by soils over a wide pH range, whereas, cations have high mobility in groundwater only under low pH conditions.

Degraffenreid and Shreve (1998) emphasised that contamination of drinking water as a result of leaking and failing landfills is of concern to public health. Chlorinated aliphatic hydrocarbons such as trichloroethylene (TCE) are common to both municipal and hazardous waste landfills and are potential contaminants of groundwater. Moreover, landfill leachate typically contains large amounts of organic carbon, nitrogen

and heavy metals and posses a low redox potential.

Carrieri and Masciopinto (1998) carried out a study of leachate of Apulia (Southern Italy) solid waste landfills to define some analytical constituents for an easy detection of groundwater pollution. They reported that NO_3^-, Cl^-, Ca^{++}, Mg^{++}, P_{tot}, SO_4^-, Na^+ and heavy metals are not suitable to identify pollution by leachate in Apulia's groundwater since they do not produce substantial changes of groundwater concentrations. K^+, NH_4^+, TOC and phenol constituents were more representative of leachate contaminations.

1.2.2. IRRIGATION WATER QUALITY PROBLEMS

Ayers and Westcot (1985) reported that irrigation water can vary greatly in quality depending upon type and quantity of dissolved salts which are present in irrigation water in relatively small but significant amounts. They originate from dissolution or weathering of the rocks and soil, including dissolution of lime, gypsum and other slowly dissolved soil materials. The suitability of a water for irrigation is determined not only by the total amount of salt present but also by the kind of salt. However, water quality or suitability for use is judged on the potential severity of problems that can be expected to develop during long-term use. These problems vary both in kind and degree, and are modified by soil, climate and crop, as well as by the skill and knowledge of the water user. Water quality problems in irrigated agriculture are :

1- Salinity :

The accumulation of salt in the crop root zone to a concentration that causes a loss in yield results in salinity problem. In irrigated areas, these salts often originate from a saline, high water table or from salts in the applied water. The accumulation of salts in the root zone to such an extent that the crop is no longer able to extract sufficient water from the salty soil solution results in a water stress for a significant period of time, and consequently reduced yields result. The reduction in water uptake results in slow rate of the plant growth. The plant symptoms are similar in appearance to those of drought such as wilting, or a darker, bluish-green colour and sometimes thicker, waxier leaves. Symptoms vary with the growth stage but more noticeable if the salts affect the

plant during the early stages of growth (Ayers and Westcot, 1985).

Rhoades (1972) indicated that the general salt effects on crop growth are generally evidenced by retarded growth , producing smaller plants with fewer and smaller leaves.

Alawi et al. (1980) evaluated the effects of irrigating a soil over a 3 year period with three waters of different qualities in terms of chemical characteristics of the soil profile. The salinity content of the waters ranged from EC_w of 3.2×10^3 to 0.55×10^3 ms /cm. They concluded that the use of these waters resulted in different soil salinity contents, e.g., values of $EC_e \times 10^3$ for the surface 30 cm ranged from 3.50 to 2.13 for the high to low salt waters, respectively. In addition, exchangeable sodium percentage (ESP) ranged from 18.4 to 24.0 in the surface 30 cm of soil, but was the highest with the use of the lowest salinity water. Sudangrass (*Sorghum sudanese*) yields increased as the salinity of the waters decreased.

Russo (1987) investigated the effect of irrigation water quality (salinity) and quantity on the yield of lettuce (*Lactuca sativa* var. "Iceberg") in a gypsiferous desert soil (Typic Torrifluvent). Irrigation water volume (Q) ranging from 0.37 to 1.3, 0.6 to 1.6, and 0.7 to 2.4 times the Class A pan evaporation (E_o) for the irrigation water salinities of C_{iw} = 1.7, 3.1 and 4.7 ms /cm, respectively, were applied via trickle irrigation. He found that soil water content and soil water pressure were strongly affected by the volume of the irrigation water and were not affected by the salinity of the irrigation water. However, soil water salinity was affected by both quality and quantity of the irrigation waters as well as by gypsum dissolution associated with the Na-Ca exchange reaction. Lettuce yield was affected by both irrigation water quality and quantity. For instance, lettuce yield slightly decreased when the saline water (C_{iw} = 4.7 ms /cm) and a relatively large volume of irrigation water (higher than about two times the class A pan evaporation) were used. However, crop yield was considerably increased when less saline waters and volumes of water higher than $Q/E_o = 1.0$ were used, moreover, there were no apparent yield reductions when the maximum water volumes of $Q/E_o = 1.3$ (C_{iw} = 1.7 ms /cm) and $Q/E_o = 1.6$ (C_{iw} = 3.1 ms /cm) were used.

2- Water infiltration rate :

An infiltration problem related to water quality occurs due to the reduction of the normal infiltration rate for the applied water or rainfall, therefore, water remains on the soil surface too long or infiltrates too slowly to supply the crop with sufficient water to maintain acceptable yields. The salinity of the water (total quantity of salts in the water) and its sodium content relative to the calcium and magnesium content are the most common water quality factors which influence the normal infiltration rate, however, both factors may operate at the same time. A high salinity water will increase infiltration but a low salinity water or a water with a high sodium to calcium ratio will decrease infiltration.

If irrigation must be prolonged for an extended period of time to achieve adequate infiltration, secondary problems may also develop such as crusting of seedbeds, excessive weeds, nutritional disorders and drowning of the crop, rooting of seeds and poor crop stands in low-lying wet spots. Furthermore, one serious side effect of an infiltration problem is the potential to develop disease and vector (mosquito) problems. In most cases, an infiltration problem related to water quality occurs in the surface few centimetres of soil and is linked to the structural stability of this surface soil and its low calcium content relative to that of sodium (Ayers and Westcot, 1985).

Rhoades (1972) indicated that the sodicity effects on soil are evidenced by puddling and by a reduced rate of water intake.

Park and O'Connor (1980) conducted laboratory determinations of saturated hydraulic conductivity and infiltration rate with four soils varying in texture from sand to clay and with five saline-sodic waters. The waters varied in total dissolved solids from 1250 to 15000 mg /l and in SAR from 16 to 57 and were representative of saline groundwaters in New Mexico. They found that the saturated hydraulic conductivities of the soils were not significantly affected by water quality if these waters were the sole source of irrigation water. Moreover, even small additions of high-quality water ("rains") to soils previously equilibrated with the saline-sodic waters significantly decreased soil permeability. However, swelling was an important mechanism in reducing soil permeability only in the clay soil. When "rain" was introduced, dispersion and short or long-distance transport of clay apparently clogged conducting pores. The results suggest that, when saline-sodic water is the dominant irrigation source and is supplemented by "rains", (1) all waters could be used on very sandy soils, (2) no saline-

sodic waters should be used on fine-textured soils, (3) slightly sodic, but not highly sodic waters could be used on medium-textured soils.

Adamsen (1989) indicated that water from deep wells in the mid-Atlantic coastal plain have high Na levels in relation to Ca and Mg and that is, the sodium absorption ratio (SAR) is high even though the water is not saline. Sodium absorption ratio is defined as :

$$SAR = \frac{Na}{(Ca + Mg)^{1/2}}$$

Where : Na, Ca and Mg are expressed as mmol /l.

Reduction in water movement in the soil results when water with a high SAR is used for irrigation due to dispersion of the clays and increased soil pH as Na replaces other cations on the exchange complex of the soil.

Costa et al. (1991) carried out a study to quantify the effects of salinization produced in four potentially irrigable soils (Barnes loam, Parshall loam, Svea loam and Williams loam) of the northern Great Plains by irrigation with seven water qualities during 21 mo of greenhouse alfalfa production in undisturbed columns. They found that as soil-extract sodium adsorption ratio (SAR_e) increased, Parshal was the soil most susceptible to dispersion. From the surface to 15 cm depth, bulk density was reduced 0.04 to 0.06 Mg m^{-3} when the highest soluble-Ca concentration water was used.

3- <u>Specific ion toxicity :</u>

Toxicity problems occur if certain constituents (ions) in the soil or water are taken up by the plant and accumulate to concentrations high enough to cause crop damage or reduced yields. The ions accumulate to the greatest extent in the areas where the water loss is greatest, usually the leaf tips and leaf edges. The degree of damage depends on the duration of exposure, concentration by the toxic ion, crop sensitivity and the volume of water transpired by the crop. For instance, the permanent, perennial-type crops (tree crops) are the more sensitive, however, the damage occurs at relatively low ion concentrations for sensitive crops. The more tolerant annual crops are not sensitive at low concentrations but almost all crops will be damaged or killed if concentrations are sufficiently high. Climate also has an effect, for example, in a hot climate or hot part of the year, accumulation is more rapid than if the same crop was grown in a cooler

climate or cooler season when it might show little or no damage. Damage is usually first evidenced by marginal leaf burn and interveinal chlorosis but great accumulation results in reduced yields (Ayers and Westcot, 1985).

Rhoades (1972) indicated that the effects of specific ion toxicity are generally evidenced by leaf burn and defoliation.

The ions of primary concern are chloride, sodium and boron. The direct absorption of the toxic ions through leaves wet by overhead sprinklers results in toxicity. Many trace elements, in addition to sodium, chloride and boron, are toxic to plants at very low concentrations.

Since chloride is not absorbed or held back by soils, it moves readily with the soil-water, is taken up by the crop, moves in the transpiration stream and accumulates in the leaves. As the chloride concentration in the leaves exceeds the tolerance of the crop, injury symptoms develop such as leaf burn or drying of leaf tissue. Normally, plant injury occurs first at the leaf tips (which is common for chloride toxicity) and progresses from the tip back along the edges as severity increases but excessive necrosis (dead tissue) is often accompanied by early leaf crop or defoliation. With sensitive crops, the accumulation of chloride in leaves from 0.3 to 1.0 percent on a dry weight basis results in these symptoms but sensitivity varies among these crops. Many tree crops show injury above 0.3 percent chloride (dry weight) [Ayers and Westcot, 1985].

Orphanos (1987) carried out two experiments to identify some of the causes of the high variability of the ratio of midrib to lamina chloride in tobacco leaves delivered to the curing plant. He found that in young tobacco plants chloride concentration was highest in the third or fourth leaf from the base of the plant, but in more mature plants (when the inflorescence began to appear) leaf chloride increased linearly from the apex to the base of the plant. The ratio of the concentration of midrib chloride to that of lamina chloride was always highest in the basal leaves, but decreased with increasing chloride concentration in the irrigation water, i.e. with increasing chloride supply more chloride went to the lamina than to the midrib per unit dry weight.

Sodium toxicity is not as easily diagnosed as chloride toxicity but clear cases of the former have been recorded as a result of relatively high sodium concentrations in the water (high Na or SAR). Typical toxicity symptoms are leaf burn, scorch and dead tissue along the outside edges of leaves in contrast to symptoms of chloride toxicity

which occur initially at the extreme leaf tip. Symptoms appear first on the older leaves, starting at the outer edges, and as the severity increases move progressively inward between the veins toward the leaf centre. An extended period of time (many days or weeks) is normally required before accumulation reaches toxic concentrations. Sensitive crops include deciduous fruits, nuts, citrus, avocados and beans but there are many others. For tree crops, as sodium in the leaf tissue exceeds 0.25 to 0.50 percent (dry weight basis) sodium toxicity results (Ayers and Westcot, 1985).

Rhoades (1972) indicated that the sodium toxicity is generally evidenced by leaf burn and defoliation.

Boron unlike sodium, is an essential element for plant growth. Chloride is also essential but in such small quantities that it is frequently classed non-essential. Boron is needed in relatively small amounts, however, it becomes toxic if present in amounts appreciably greater than needed. For some crops, if 0.2 mg /l boron in water is essential, 1.0 to 2.0 mg /l may be toxic. Boron toxicity can affect nearly all crops but, like salinity, there is a wide range of tolerance among crops. Boron toxicity symptoms normally show first on older leaves as a yellowing, spotting, or drying of leaf tissue at the tips and edges. As more and more boron accumulates with time, drying and chlorosis often progress toward the centre between the veins (interveinal) [Ayers and Westcot, 1985].

Richards (1954) pointed out that boron is essential to the normal growth of all plants but the quantity required is very small. A deficiency of boron produces striking symptoms in many plant species. Boron is very toxic to certain plant species, however, the concentration that will injure these sensitive plants is often approximately that required for normal growth of very tolerant plants. For instance, lemons show definite and , at times, economically important injury when irrigated with water containing 1.0 mg /l of boron, while alfalfa will make maximum growth with 1.0 to 2.0 mg /l of boron.

4- <u>Miscellaneous effects :</u>

Several other problems related to irrigation water quality occur with sufficient frequency for them to be specifically noted. These include high nitrogen concentrations in the water which supplies nitrogen to the crop and may cause excessive vegetative growth, lodging and delayed crop maturity; unsightly deposits on fruit or leaves due to overhead sprinkler irrigation with high bicarbonate water, water containing gypsum or

water high in iron; and various abnormalities often associated with an unusual pH of the water.

The normal pH range for irrigation water is from 6.5 to 8.4. An abnormal value is a warning that the water needs further evaluation. For example, irrigation water with a pH outside the normal range may cause a nutritional imbalance or may contain a toxic ion. Low salinity water (EC_w < 0.2 ds /m) sometimes has a pH outside the normal range since it has a very low buffering capacity. Such water normally causes few problems for soils or crops but is very corrosive and may rapidly corrode pipelines, sprinklers and control equipment. However, any change in the soil pH caused by the water will take place slowly since the soil is strongly buffered and resists change. An adverse pH may need to be corrected , if possible, by the introduction of an amendment into the water, but this will only be practical in a few instances. For example, lime is commonly applied to the soil to correct a low pH but gypsum has little or no effect in controlling an acid soil problem apart from supplying a nutritional source of calcium. However, gypsum is effective in reducing a high soil pH (pH greater than 8.5) caused by high exchangeable sodium. In addition, sulphur or other acid material may be used to correct a high pH. The greatest direct hazard of an abnormal pH in water is the impact on irrigation equipment.

Several other problems are related to irrigation water quality such as the deterioration of equipment due to water-induced corrosion or encrustation. This problem is most serious for wells and pumps, but in some areas, a poor quality water may also damage irrigation equipment and canals. In areas where there is a potential risk from diseases such as malaria, schistosomiasis and lymphatic filariasis, disease vector problems must be considered along with other water quality-related problems. Suspended organic as well as inorganic sediments cause problems in irrigation systems through clogging of gates, sprinkler heads and drippers. Moreover, they can cause damage to pumps if screens are not used to exclude them. Furthermore, more commonly, sediments tend to fill canals and ditches and cause costly dredging and maintenance problems. Finally, sediment tends to reduce further the water infiltration rate of an already slowly permeable soil (Ayers and Westcot, 1985).

1.2.3. WATER QUALITY PARAMETERS AND EVALUATION

Owens et al. (1994) conducted a study to determine groundwater NO_3-N levels following a change in N source from fertiliser to a legume in a grass-pasture grazed by beef cattle. They pointed out that nitrogen in groundwater was present mainly in the NO_3-N form and concentrations increased and reached levels that were usually in excess of 10 mg N /l throughout the 5 year period of fertiliser application. Changing from N fertiliser to legume N resulted in a rapid drop in the NO_3-N concentrations in groundwater during a 2 year period. The decrease in NO_3-N levels occurred from 17.7 to 9.3 mg N /l in a tall fescue-alfalfa area and from 11.2 to 2.7 and from 8.3 to 3.6 mg N /l in two orchard-grass-alfalfa areas. During the remainder of the 10 year period, NO_3-N concentrations declined to levels similar to those before N fertilisation.

Richards et al. (1996) used the Heidelberg College Co-operative Private Well Testing Program to study the relationships of concentrations of nitrate and two herbicide groups to factors that are generally believed to reflect vulnerability of a well to contamination. They concluded that the median nitrate concentration was 0.07 mg /l, however, 3.4 % of wells exceeded the drinking water standard of 10 mg /l and 23 % exceeded 1.0 mg /l. Nitrate, triazine and acetanilide concentrations are higher in wells that are shallower, older, dug or driven; located close to cropland, feedlots, or chemical mixing sites; or located in sandy soils.

Blevins et al. (1996) designed a field experiment to trace and isolate the amount of a single application of N fertiliser lost to a glacial-till aquifer and runoff from a 400 m^2 corn (*Zea mays* L.) plot with bromide (Br^-) and isotopically labelled (^{15}N) fertiliser. They pointed out that the claypan did not affect the transport of water and NO_3-N to the saturated zone. Labelled-N fertiliser accounted for as much as 8.6 mg /l of the NO_3-N (as N) in groundwater but only in the top 1.0 to 2.0 m of the saturated zone. Because of the increase in groundwater concentrations of labelled NO_3-N after two growing seasons, rotation of crops that requiring small N imputs could be expected to limit the cumulative effect of large annual fertiliser applications on groundwater. The slow movement of groundwater and the apparent chemical stability of NO_3-N in shallow groundwater may explain the possibility of shallow uncased wells in large parts of the Midwest (Missouri, USA) to have large NO_3-N concentrations more than deeper cased wells.

Kent and Spycher (1994) showed that the low dissolved metal concentrations

in most unpolluted groundwater have no significant effect on the design of groundwater investigations or remediation systems. Exceptions to this are iron and to a lesser extent, manganese, which can precipitate in wells, pumps, piping, or treatment systems. Although iron is ubiquitous in many rocks and soils, its solubility in water (at ambient temperature and neutral pH ranges) as inorganic species is generally low, in addition, the concentration of iron in most groundwaters rarely exceeds a few milligrams per litre. However, iron concentrations, in the form of organic complexes and/or colloids, can become quite high under reducing conditions in waters having a high organic matter content. For example, iron concentrations in excess of 40 mg /l occurred in the recovered groundwater as a result of the combination of the organic contaminants and high iron content in groundwater at an industrial facility in the north-eastern United States.

Kent and Spycher (1994) indicated that groundwater normally contains a higher concentrations of dissolved carbon dioxide gas than does surface water. As water is brought to the surface, and the pressure decreases from hydrostatic to atmospheric, some of the dissolved gas comes out of solution, resulting in increase in the pH of the water. The dissolution of carbon dioxide in precipitation results in the formation of carbon acid , consequently the pH of precipitation waters is mildy acidic even in areas having unpolluted air.

The quality of water for irrigation can be evaluated according to its parameters and related problems. Different guidelines for evaluating water quality were introduced by several authors.

Richards (1954) reported that in classification of irrigation waters, it is assumed that the water will be used under average conditions with respect to soil texture, infiltration rate, drainage, quantity of water used, climate and salt tolerance of crop. Large deviations from the average for one or more of these variables may make it unsafe to use what, under average conditions, would be a good water; or may make it safe to use what, under average conditions, would be a water of doubtly quality. The characteristics of an irrigation water that appear to be most important in determining its quality are :

1- Total concentration of soluble salts :

The total concentration of soluble salts in irrigation waters can be adequately

expressed for purposes of diagnosis and classification in terms of electrical conductivity which is useful because it can be readily and precisely determined. Nearly all irrigation waters that have conductivity values less than 2250 micromhos /cm have been used successfully for a considerable time. Waters of higher conductivity are used occasionally, however, crop production, except in unusual situations, has not been satisfactory. Saline soils are those with conductivity of the saturation extract greater than 4 millimhos /cm, or 4000 micromhos /cm.

2- Relative proportion of sodium to other cations :

The soluble inorganic constituents of irrigation waters react with soils as ions rather than as molecules. The principal cations are calcium, magnesium and sodium, with small quantities of potassium ordinarily present. The principal anions are bicarbonate, sulphate and chloride, with fluoride and nitrate occurring in low concentrations. If the proportion of sodium is high, the alkali hazard is high, and, conversely, if calcium and magnesium predominate, the hazard is low. Alkali soils are formed by accumulation of exchangeable sodium and are often characterised by poor tilth and low permeability.

3- Concentration of boron or other elements that may be toxic :

Boron is a constituent of practically all natural waters and the concentrations varying from traces to several parts per million. It is essential to plant growth , but is exceedingly toxic at concentrations only slightly above optimum. It necessary to consider this element in assessing water quality due to the occurrence of boron in toxic concentrations in certain irrigation waters.

4- Under some conditions, the bicarbonate concentration as related to the concentration of calcium plus magnesium :

In waters containing high concentrations of bicarbonate ion, there is a tendency for calcium and magnesium to precipitate as the soil solution becomes more concentrated.

Rhoades (1972) indicated that the suitability of an irrigation water needs to be evaluated on the basis of criteria indicative of their potentials to create soil conditions hazardous to crop growth or animals or humans consuming those crops. In addition, the specific conditions under which irrigation water will be used include grown crops, soil

properties, irrigation management, cultural practices, and climatic conditions. The prevailing criteria of irrigation water quality and their associated potential hazards to crop growth are :

1- Salinity :

The general salt effects on crop growth thought to be largely osmotic in nature and related to total salt concentration rather than to the individual concentrations of specific salt constituents.

2- Sodicity :

Sodicity is the effect of an excessive amount of exchangeable sodium in the soil on soil permeability, soil structure deterioration, and a direct toxic effect of exchangeable sodium in plants specifically sensitive to sodium.

3- Toxicity :

Toxicity is the specific ion effects of solutes (other than sodium) of a nutritional nature, especially those of chloride and boron.

Abdel Hamid and Hamdi (1974) carried out a study to find out a Suitability Index of the main drain waters for irrigation purposes including soil-plant characteristics. They suggested to give rating values for irrigation water characteristics, soil texture, and salt tolerance of crops so that the sum of all rating indexes does not exceed a certain value, otherwise a harmful effect might arise. The present assumption proposed the following rating indexes :

For SAR :

1 for < 10 SAR.
2 for 10 - 18 SAR.
3 for 18 - 26 SAR.
4 for > 26 SAR.

For salinity :

1 for < 750 micromhos /cm.
2 for 750 - 1750 micromhos /cm.
3 for 1750 - 3000 micromhos /cm.
4 for > 5000 micromhos /cm.

For pH :

1.0 for pH 7.0 - 7.5

1.5 for pH 7.5 - 8.0

2.0 for pH 8.0 - 8.5

2.5 for pH 8.5 - 9.0

3.0 for pH > 9.0

For RSC :

1 for RSC < 1.25

2 for RSC 1.25 - 2.50

3 for RSC > 2.50

For soil texture :

4.0 for clayey textured soils.

3.5 for clay loamy textured soils.

3.0 for loamy textured soils.

2.5 for sandy loam textured soils.

2.0 for sandy textured soils.

For plant tolerance :

1 for salt tolerant crops.

2 for semi-tolerant crops.

3 for sensitive crops.

The results of experiments conducted using six different water qualities similar in nature to those of drain waters, and adopting the aforementioned proposed rating index, showed that saline irrigation waters can be safely used as long as the sum of all rating indexes of irrigation water characteristics, soil texture and plant tolerance does not exceed 13.

Ayers and Westcot (1985) indicated that the prediction that a water quality-related problem will occur requires evaluation of the potential of the water to create soil conditions that may restrict its use or that may require the use of special management techniques to maintain acceptable yields. The four problem categories previously discussed (salinity, infiltration, toxicity and miscellaneous effects) are used for evaluation. Water quality problems, however, are often complex and a combination of problems may affect crop production more severely than a single problem in isolation. The more complex the problem, the more difficult it is to formulate an economical management programme for solution.

Salem et al. (1995) showed that according to European Community standards, drinking water must contain no more than 50 mg /l of nitrate, however, a value of 25 mg /l is highly recommended.

Lesage (1991) pointed out that the characterisation of groundwater contaminated with industrial wastes of landfills is a very difficult task since several hundred different chemicals which were either products or by-products are co-disposed over a very long period of time.

1.3. BIOREMEDIATION TREATMENT OF AMMONIUM AND NITRATE CONTAMINATED GROUNDWATER

With the limited sources of fresh waters and the increase in the population, there is essential need for treating the contaminated groundwater in order to be used as a fresh source of water. The contaminated groundwater can be treated by different processes such as physical, chemical and biological processes. At present, the bioremediation treatment is the most common treatment for the contaminated groundwater. One of the biological processes for the removal of ammonium is the oxidation of ammonium to nitrite and nitrate by autotrophic bacteria (*Nitrosomonas* and *Nitrobacter*). In addition, the bioremediation treatment includes the use of trees and grass to uptake the contaminants into their tissues and remove them from the environment, and this is referred as phytoremediation.

Burton and Watson-Craik (1998) indicated that ammonia is both a necessary requirement for the growth of bacteria and for the decomposition processes which they control. Moreover, it is a toxic by-product of the landfill degradation processes.

Azov and Tregubova (1995) showed that the nitrification processes in stabilisation reservoirs have a major role in improving water quality. As the wastewater effluents which enter the reservoir usually have a substantial concentration of ammonia, the oxidation of ammonia to nitrates through nitrification processes increases the options of using the water for different purposes.

Cardenas and Molof (1970) indicated that the removal kinetics of waste compounds which have been discharged to a receiving stream are of special interest to wastewater engineers. Of the compounds found in wastewater stream, only a few are amenable to continuous chemical analysis such as ammonia.

Jowett and McMaster (1995) developed a new type of single-pass aerobic biofilter as an alternative to the conventional septic tile bed and for treatment of wastewater in general. They indicated that the latest field trial removes 97.8 % BOD_7, 96.1 % TSS, and 99.5 % fecal coliform bacteria with 12 to 16 °C wastewater loaded at 49 cm /d. In laboratory column experiments, removal of fecal coliforms averages > 99.99 % at 80 cm /d loading, and 99.999 % at 10 cm /d after a 10 to 14 d acclimatisation period. Ammonium is thoroughly oxidised to NO_3-N with typically < 2.5 mg /l NH_4-N

in the effluent.

Salem et al. (1995) compared the performance of three membrane processes (Donnan dialysis, electrodialysis and electrodeionization) using an ADS-Morgane anion-exchange membrane, to remove nitrate from drinking water. They reported that the three membrane processes considered are able to greatly decrease nitrate content in polluted water, as the extraction ratios are higher than 80 % in all cases. With the electodialysis and electrodeionization techniques, the extraction rate of nitrate was faster than with Donnan dialysis, and a deionised water of high purity was obtained. A nitrate elimination ratio of 99 % has been reached with electrodeionization. In Donnan dialysis process, nitrate, bicarbonate and sulphate anions are substituted by chloride anions whose permitted level is 250 mg /l, five times higher than the permitted nitrate level. The efficiency of these techniques can be improved by using anion exchange membranes with a higher nitrate selectivity.

1.3.1. BIOREMEDIATION TECHNOLOGIES

Alexander (1994) stated that bioremediation of contaminated sites is a new field of endeavour, and many new or altered technologies are appearing. The target of bioremediation is to degrade organic pollutants to concentrations that are either undetectable or, if detectable, to concentrations below the limits established as safe or acceptable by regulatory agencies. Bioremediation is widely used for the destruction of chemicals in soils, groundwater, wastewater, sludges, industrial-waste systems, and gases. A variety of different technologies and procedures are currently being used, and a number of new and promising approaches have been suggested or have reached advanced stages of development. Some of these technologies are *in situ* treatments, in which soil is not removed from the field or groundwater is not pumped for aboveground treatment. The advantage of *in situ* bioremediation is the relatively low cost but the disadvantage is being less subject to rigorous control. In addition, other bioremediation technologies require removal of the contaminated material in some manner from its original location. Such removals increase the costs modestly or appreciably, but the processes are more subject to control.

1.3.1.1. DENITRIFICATION

Harper et al. (1996) emphasised that the biological nitrification-denitrification process is considered to be one of the most promising and practical methods of treating high ammonia nitrogen leachates. A single-sludge, nitrification-denitrification process is capable of removing large concentrations of ammonia and total nitrogen from landfill leachates.

Hanson et al. (1994) measured denitrification in riparian forests with upland to wetland transition zones (moderately well drained and somewhat poorly drained soils) and red maple (*Acer rubrum* L.) swamps (poorly and very poorly drained soils) on two sides of a stream. They summarised that comparison of measured denitrification rates with estimates of groundwater NO_3-N loading suggested that denitrification may have removed up to 50 % of the groundwater NO_3-N that entered the enriched site.

Weier et al. (1994) conducted a field study on a Hord silt loam (Pachic Haplustoll) in central Nebraska by installing plastic (PVC) cylinders (28.7 cm diam. by 1.8 m long) in soil to a depth of 1.2 m and irrigating with 17.1 cm of water containing 30 mg /l isotopically enriched (76.6 atom % ^{15}N) KNO_3-N equivalent to 51.8 kg N /ha. They found that with the addition of ethanol and NO_3-N solution, biological denitrification was increased from the surface of the soil columns, from within the soil columns at four depths in the soil profile, and from the incubated intact soil cores. Therefore, the prevention of groundwater contamination by high NO_3-N irrigation water through microbial denitrification, with ethanol as the C source, remains a possibility. However, this approach to bioremediation of an existing problem should only be an interim solution, as better N management practices are the preferred and most efficient long-term solution to preventing groundwater contamination.

Becker et al. (1997) investigated the process of leachate denitrification by populations of nitrifying and denitrifying bacteria. Leachate, derived from a local municipal landfill site, was nitrified in a continuously operating packed-bed biofilm reactor and thereafter denitrified in an activated sludge bioreactor. They found that the ammonium concentrations decreased to 1 - 5 mg /l during nitrification under continuous operating conditions. Increased ammonium concentrations after nitrification correlated with a decrease in the efficiency of nitrogen elimination by up to 45 % due to the build-up of high concentrations of nitrite.

Bae et al. (1997) treated leachates from a municipal solid waste landfill by

anaerobic filter and two-stage activated sludge for the removal of ammonia. They concluded that with the above treatment system, 1400 ~ 1800 mg /l of NH_4-N was completely removed, leaving 200 mg /l of nitrate nitrogen. In addition, 4000 ~ 7000 mg /l of COD in the raw leachate was reduced to 150 ~ 200 mg /l.

Hippen et al. (1997) demonstrated that with the aid of the operating results and the more accurate studies carried out by the authors concerning the biological pre-treatment of the leachate treatment plant of the landfill Mechernich, it could be stated that within the nitrification step under aerobic, but oxygen limited conditions, a considerable part of the nitrogen load is eliminated by means of the processes of aerobic deammonification.

Onay and Pohland (1998) designed a three reactor system to stimulate the landfill environment and to investigate the potential for *in situ* of nitrogen removal in dedicated nitrification / denitrification zones. They pointed out that both separate and combined reactor operation with internal leachate recycle provided 95 % nitrogen conversion. In contrast, combined reactor operation with single pass leaching provided a conversion efficiency per cycle ranging between 30 - 52 % for nitrification and 16 - 25 % for denitrification, thereby indicating the efficacy of using the landfill itself for attenuation of leachate ammonia nitrogen concentrations to levels acceptable for ultimate discharge. The efficiency of nitrogen conversion was dependent on the operational stages.

1.3.1.2. PHYTOREMEDIATION OF INORGANIC CONTAMINATION

Cunningham et al. (1997) reported that phytoremediation, or the use of plants to remediate contaminated soils and water environments, has recently become an area of intense study. The traditional methods of remediating contaminated soils and water, such as excavation or combustion, are environmentally disruptive and quite expensive. While the perceived advantage of bioremediation is the often prohibitive cost of effective engineering approaches. For example, phytoremediation may provide a cost-effective remediation technique for some contamination situations. In addition, it may make it possible to treat most contaminants in situ. Phytoremediation is the use of green plant-based systems to remediate contaminated soils, sediments, and water. Relative to many traditional remediation engineering techniques, phytoremediation is a fledgling technology intended to address a wide variety of surficial contaminants.

Phytoremediation targets currently include contaminating metals, metalloids, petroleum hydrocarbons, pesticides, explosives, chlorinated solvents, and industrial by-products. However, phytoremediation has inherent limitations in that plants are living organisms with specific oxygen, water, nutrient and pH limits that must be maintained.

Salt et al. (1998) demonstrated that contaminated soils and waters pose a major environmental and human health problem, which may be partially solved by the emerging phytoremediation technology. This cost effective plant-based approach to remediation takes advantage of the remarkable ability of plants to concentrate elements and compounds from the environment and to metabolise various molecules in their tissues. Toxic heavy metals and organic pollutants are the major targets for phytoremediation. Phytoremediation is currently divided into the following areas :

1- Phytoextraction : the use of pollutant accumulating plants to remove metals or organics from soil by concentrating them in the harvestable parts.

2- Phytodegradation : the use of plants and associated micro-organisms to degrade organic pollutants.

3- Rhizofiltration : the use of plant roots to absorb and adsorb pollutants, mainly metals, from water and aqueous waste streams.

4- Phytostabilization : the use of plants to reduce the bioavailability of pollutants in the environment.

5- Phytovolatilization : the use of plants to volatilize pollutants.

6- The use of plants to remove pollutants from air.

Phytoextraction and hyperaccumulators are the most common techniques of phytoremediation for inorganics contamination.

Phytoextraction:

Contamination can be reduced as a result of plant uptake into the tissue where it can be further degraded to innocuous substances, or removed from the site. For example, plants can be used to extract contaminants from the environment, a process referred to as phytoextraction. Phytoextraction involves the use of plants to extract contaminants from the environment. The term was originally applied almost exclusively to heavy metals in soils but has since come to apply to many other materials in other media as well. The phytoextraction R & D community involved with inorganics has

gradually evolved into two groups. The first group uses phytoextraction for remediation purposes (primarily targeting Pb and radionuclides with some efforts on Cr, As, and Hg); and the second group targets inorganics with intrinsic economic value (primarily Ni, some Cu and a few with precious metals) (Cunningham et al., 1997).

Hyperaccumulators :

Hyperaccumulating plants, can take up, translocate and tolerate shoot concentrations of heavy metals in excess of 0.1 % Ni, Co, Cu, Cr, Pb or 1.0 % Zn on a dry weight basis (Baker and Walker, 1990). The remarkable advantage of these plants, which often evolved on metalliferous outcroppings, not only their high levels of accumulation and tolerance, but also their nearly insatiable desire to concentrate these elements from even "normal" soil. Many of these species seem to hyperaccumulate only one metal while most sites have mixed metal contaminants. In addition, these plants prove that biological systems can be developed with plants maintaining up to 4 % metal in their tissues without significant yield decreases (Cunningham et al., 1997).

1.3.1.2.1. PHYTOREMEDIATION OF NITROGEN CONTAMINATED WATER USING GRASS

Grass has the biggest potential dry matter of any crop in the United Kingdom. Under ideal conditions the national average yield could be about 12 tonnes of dry matter per hectare per year, but at present it is only about 4 tonnes /ha of dry matter per year. This ranges from about 2 tonnes in the hills and uplands to about 10 tonnes in intensively fertilised lowland grass. The grass plant survives grazing and cutting because the growing point stays close to the ground for most of the year, during this vegetative phase the growing point continually produces leaves and buds. Grass responds linearly to increasing amounts of nitrogen up to about 350 kg per hectare. However; from then on the response levels off until, at about 500 kg per hectare; there is little or no extra growth. Each kg of nitrogen applied up to the 350 kg per hectare mark is producing about 25 kg of dry matter in an all-ryegrass sward (Craven et al., 1981).

The advantages of using grass for the present study are :

1- The soil and climatic conditions of the site of study are suitable for the growth of grass.

2- It is tolerant of variations in nutrient balance.

3- It is surviving cutting.

4- It tolerates and utilises high level of nitrogen.

5- It has high dry matter production.

1.4. OBJECTIVES OF THESIS

The Ardeer site of Nobel Enterprises has been used for the manufacture of a range of chemicals and explosives such as Nitro-cellulose, Nitro-glycerine, Dyestuffs and Nylon, and their associated acids, predominately Sulphuric and Nitric acids. A small part of the site was also set aside as a licensed landfill facility. As a result of some of these activities localised contamination has occurred. The particular interest of this study is the nitrogen contamination of the groundwater associated with the areas of the site where the groundwater has been contaminated by ammonium and nitrate. The objectives of the current study were mainly as follows : (1) to investigate the distribution and concentration of ammonium-N, nitrate-N and other contaminants such as chloride, sodium, potassium, calcium, magnesium, and iron in the groundwater at the Ardeer site, (2) to identify the sources of nitrogen contamination, (3) to evaluate the groundwater quality parameters, and (4) to assess the effects of groundwater composition on nitrification in soil. In addition, another important objective was also to look at the possibility of bioremediation treatment of the groundwater. The bioremediation treatment being considered is by pumping well water to the surface, irrigating the vegetation, and harvesting the vegetation [as vegetation such as perennial ryegrass (*Lolium perenne* L.) uses ammonium and nitrate as a fertiliser before it leaches down in soil profile]. This bioremediation treatment could be applied to other circumstances in which groundwater is contaminated.

This thesis covers the following studies :

1- Ammonium analysis in soil and water including the determination of low levels of ammonium (< 0.1 mg N / l) in groundwater and the colorimetric analysis of highly coloured groundwater samples.
2- An investigation of groundwater quality and soils at an contaminated industrial site.
3- Bioremediation treatment of the ammonium and nitrate contaminated groundwater using natural soil and vegetation and using soil incubation and pot experiments.

The work presented in this thesis has been divided to 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 objectives of the work to be undertaken, and the objectives 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 method development and assessment. Analytical studies were carried out for the optimisation of the method of ammonium-N determination on a Technicon Autoanalyzer II system. The purpose of this study was to optimise the reagent concentrations, the pH of the reaction, the temperature of the manifold water bath, determine the absorbance spectrum of the indophenol product, and evaluate the optimised method for the determination of ammonium in soil extracts and water samples in terms of the precision and sensitivity. In addition, analytical studies were accomplished to develop a reliable Technicon Autoanalyzer method to determine low levels of ammonium in groundwater (< 0.1 mg /l). Further analytical studies were undertaken to develop a method for decolorisation which can cope with highly coloured groundwater samples prior to colorimetric analysis.

Chapter four is devoted to describe the investigation of groundwater quality and soil at the site. Groundwater surveys were carried out with the following aims :

1- To investigate the distribution and concentration of ammonium-N, nitrate-N and other contaminants such as chloride, potassium, sodium, calcium, magnesium and iron in the groundwater in the Ardeer site.
2- To evaluate the groundwater quality parameters and their seasonal variations over a period of time.
3- To identify the sources of nitrogen contaminated groundwater for treatment.

In addition , a soil survey was carried in selected areas where soils had been restored to characterise the soil and to evaluate the soil contamination due to toxic metals, in order to choose soil samples suitable for nitrification incubation experiment

and pot experiment.

Chapter five deals with nitrification experiments. The nitrification experiment was carried out in order to assess the effects of groundwater composition on nitrification in soil using four soil samples from the site and two control soils (natural garden soils).

Chapter six is devoted to clean up the contaminated groundwater by irrigating ryegrass with the ammonium and nitrate contaminated groundwater to remove ammonium and nitrate (as ryegrass use ammonium and nitrate as a fertiliser). This study was carried out with the following objectives :

1- To determine the influence of ammonium and nitrate contaminated groundwater on the growth of the perennial ryegrass (*Lolium perenne* L.) and soil.
2- To evaluate the efficiency of the perennial ryegrass (*Lolium perenne* L.) in the remediation of high ammonium and nitrate groundwater.

CHAPTER 2

MATERIALS AND METHODS

This chapter is devoted to provide a brief introduction and description of the apparatus, reagents, preparation of reagent solutions, analytical procedures for determining concentrations in solution, and calculation of concentrations in the materials analysed. Some of these methods are routine published methods.

2.1. CLEANING OF GLASSWARE AND PLASTICWARE

Glassware and plasticware should be cleaned physically and chemically and then rinsed free of all cleaning agents. Initial physical cleaning and rinsing process includes soaking in an appropriate chemical cleaning agent, rinsing in tap water and final rinsing in deionised water.

CARE OF GLASSWARE AND PLASTICWARE :

All glassware and plasticware selected for use were examined to ensure that it was free of chips and scratches since the surface scratching of both glass and plastic may make the surface very difficult to clean effectively.

Before washing, a suitable solvent such as ethanol and a tissue or cotton wool were used to remove all ink marks and previous labels. Self adhesive labels were avoided as they were very often difficult to remove effectively, particularly after prolonged contact.

After use the glassware was rinsed with warm tap water immediately. If the soil or solutions is allowed to dry onto surfaces this might render them more difficult to clean subsequently.

PHYSICAL WASHING :

Glassware was cleaned in warm water using a detergent such as a Teepol and a test tube brush if needed. Care was taken to avoid scratching glassware during this process. Following this stage glassware appeared to be clean both inside and outside. Then the glassware was rinsed 5 times with warm tap water.

CHEMICAL CLEANING :

The glassware which was already physically clean was soaked in chemical cleaning agent.

The cleaning solution can be used depending on the nature of the chemical contamination and the type of analysis.

2 % of Decon 90 solution (Decon Laboratories Limited) is a general purpose soaking solution which has a good rinsing properties. Glassware was soaked in a container of this solution for overnight. The flasks etc. were completely filled with and immersed in the solution to ensure a good effectiveness. This is an alkaline detergent solution and may not remove some alkali insoluble materials. In addition, it is also unsuitable for cleaning metallic material which it will dissolve and for which Decon Neutral should be used.

Provided the glassware was physically cleaned before chemical cleaning the chemical cleaning agent can be used repeatedly for several weeks until it becomes reduced in its effectiveness or becomes heavily contaminated. Unfortunately there is no easy way to tell when this has occurred except that blank values may increase.

RINSING :

Glassware was rinsed 5 times with warm tap water to ensure complete removal of the chemical cleaning agent. This was followed by rinsing twice with deionised water and finally drying in an oven at 70 °C.

Care was taken to ensure that the oven dried glassware did not become contaminated e.g. by dust or contact with dirty surfaces.

2.2. PURIFICATION OF THE FILTER PAPER TO REMOVE NITROGEN CONTAMINATION

The filter papers were purified as described in Shah method (1988).

PROCEDURE :

0.5 M sulphuric acid was prepared from analar grade concentrated acid using nitrogen - free deionised water. Each filter paper was folded separately into a clean and dry plastic funnel. Then 50 cm^3 of 0.5 M sulphuric acid was filtered through each filter paper in two equal successive portions of 25 cm^3 each. This was followed by rinsing the acid washed filter papers 3 times with deionised water to wash away any acid left in the filter paper. Care was taken to ensure that the filter papers were made acid - free. Therefore, litmus test paper was used for this purpose. Then the washed filter papers along with funnels were dried for 4 hours in the oven at 70 ^oC before using for filtration.

2.3. MEASUREMENT OF SOIL pH

The pH of soil samples measured in the laboratory varies according to the sampling and treatment. The principal variables affecting the measurement of the pH are the field variation, predrying of the soil, the amount of grinding, the carbon dioxide content, the soluble salt content and the soil : water ratio used.

The pH was measured in soil according to MAFF /ADAS method No. 32 (1986). Soil pH was measured in a 1 : 2.5 soil : deionised water mixture by a combined glass / reference electrode. The pH meter (Mettler - Model : Delta 320) was first standardised with buffer solutions of pH 7.0 and 4.0. The buffer solutions were prepared by dissolving buffer tablets in 100 ml deionised water.

A 20 g of air dried soil passed through a 2 mm sieve, was taken into 100 ml glass bottle and 50 ml of deionised water was added to the bottle. The bottle was shaken for half an hour on an end - over - end shaker. The soil suspension was stirred by swirling the electrode slightly and the pH value was recorded when the reading stabilised.

2.4. MEASUREMENT OF WATER pH

The pH was measured in the water samples by a combined glass / reference electrode as follows :

The pH meter (Mettler - Model : Delta 320) was first standardised with buffer solutions of pH 7.0 and 4.0. The buffer solutions were prepared by dissolving buffer tablets in 100 ml deionised water.

20 ml of the water sample was placed in 50 ml beaker. The water sample was stirred by swirling the electrode slightly and the pH value was recorded when the reading stabilised.

2.5. MEASUREMENT OF THE ELECTRICAL CONDUCTIVITY (EC) OF SOIL

Soil conductivity was measured in a 1 : 2.5 soil : deionised water mixture using a Jenway 4070 conductivity meter. It was measured in the same way as pH. 20 g of air dried soil passed through a 2 mm sieve, was taken into 100 ml glass bottle and 50 ml of deionised water was added to the bottle. The bottle was shaken for half an hour on an end - over - end shaker. The soil suspension was filtered through a Whatman No. 1 filter paper and the filtrate was collected. The conductivity electrode was swirled in the filtered solution and the conductivity value was recorded when the reading stabilised.

2.6. MEASUREMENT OF THE ELECTRICAL CONDUCTIVITY (EC) OF WATER

The EC was measured in the water sample before the pH since the pH electrode contains KCl solution and the pH electrode was designed to leak potassium which may contaminate the water sample.

The EC was measured in the groundwater samples using a Jenway 4070 conductivity meter. The conductivity electrode was swirled in the water sample and the conductivity value was recorded when the reading stabilised.

2.7. DETERMINATION OF MOISTURE CONTENT OF SOIL

All air dried soils contain water which is very strongly held by the soil particles. The weight of this hygroscopic water depends partly on the relative humidity and temperature of the atmosphere. Although the moisture content of air dry soil is of little interest in itself, it is necessary to know precisely what the moisture content is. Although soil analysis are carried out using air dry soil, results are reported on an oven dry basis (110 °C). Therefore, by doing this conversion, it is possible to compare results obtained at different times and in different laboratories on soils which may have been air - dried at slightly different temperatures (usually 30 - 35 °C).

Moisture content was determined in vitreosil (silica) basins because the soil samples will later be used to measure loss on ignition. Vitreosil (silica) basins were washed, cleaned and then left in the electric muffle furnace (Gallenkamp Muffle Furnace) at 600 °C for 2 hours. Then the furnace was switched off to cool down. Then the vitreosil (silica) basins were placed in the oven at 110 °C for 2 hours to dry. They were cooled in a desiccator for 30 minutes and weighed. 10 g of fresh soil passed through a 2 mm sieve was weighed, in triplicate, into each basin which was then placed for 24 hours in an oven at 110 °C, cooled in a desiccator for 30 minutes and reweighed. The samples were retained for the measurement of loss on ignition. The percent moisture content was determined in an oven dry basis as follows :

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

2.8. DETERMINATION OF SOIL ORGANIC MATTER BY LOSS ON IGNITION

Ignition of the soil at high temperature (e.g. 700 °C) will result in loss of weight due to loss of organic matter and loss of combined water (calcareous soil will also lose carbonate). However, the ignition at lower temperature (e.g. 400 °C) will

reduce the error due to loss of combined water, but will require a longer time for complete combustion of the organic matter.

Following the determination of moisture content, the silica basins were placed in the electric muffle furnace at 600 °C for 8 hours. Then the furnace was switched off to cool. The basins were transferred to an oven for 2 hours at 110 °C, then cooled in a desiccator for 30 minutes and reweighed. The organic matter content was calculated as a percentage loss on ignition as follows :

$$\% \text{ Loss on ignition} = \frac{\text{Weight of oven dry soil - Weight of ignited soil}}{\text{Weight of oven dry soil}} \times 100$$

2.9. DETERMINATION OF THE SOIL MOISTURE CONTENT AT -0.5 BAR SOIL MOISTURE POTENTIAL

Determination of moisture content at -0.5 bar soil moisture potential was carried out with the pressure plate apparatus (Manifold # 750 Series, Moisture Equipment Company, Santa Barbara, California, USA). The three replicate samples of fresh soil were placed undisturbed on the plate in plastic rings, flooded with about 1.0 cm depth of deionised water, covered with a plastic sheet to avoid the evaporation of water, and allowed to soak for 24 hours. The excess of water was then removed from the plate which was then placed in the pressure plate apparatus and the pressure was 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. Then the samples were transferred to a labelled and weighed silica basin. The silica basin along with the soil sample was weighed. The percentage moisture content was determined on an oven dry basis as described in section 2.7.

2.10. SOIL MECHANICAL ANALYSIS

Particle size analysis for the determination of the textural class was modified from the ADAS method (1981) and the modification of Khan (1987).

2.10.1. REAGENTS

(a)- Hydrogen peroxide 30 %

(b)- Dispersing reagent (calgon) :

50.0 g of sodium hexametaphosphate plus 7.0 g of sodium carbonate (anhydrous) were dissolved in deionised water and diluted into 1 litre.

(c)- Silicon antifoaming agent :

1 ml of 30 % aqueous emulsion was diluted into 100 ml deionised water.

(d)- 2 M HCl :

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

2.10.2. PROCEDURE

2.10.2.1. DISPERSION

The air dried soils were passed through a 2 mm sieve. 10 g of each soil was weighed into a 600 ml beaker. A 30 ml deionised water was added to the beaker. Then 20 ml of hydrogen peroxide (30 %) and 2 drops of antifoaming agent were added to each beaker. The initial reaction was allowed to subside for 20 minutes. The beakers were then gently heated on a steam bath and the contents were stirred occasionally with a glass rod. The heating was continued until the reaction ceased. The beakers were cooled and a further 10 ml of hydrogen peroxide (30 %) was added washing down the sides of the beaker and heating continued until the reaction ceased completely. 30 ml of hydrogen peroxide (30 %) was sufficient for most of the soils except those high in organic matter where additions were continued until no reaction was observed.

For soils containing calcium carbonate, approximately 2 M HCl was added dropwise and the contents of the beaker were stirred continuously until the effervescence ceased. For all soils a further 10 ml of 2 M HCl was added providing a

dispersion of soil in approximately M/5 HCl. The beakers were stirred at intervals during an hour and then the soil was allowed to settle. The soil suspension was filtered through a Whatman No. 50 filter paper under suction. The soil was washed in the beaker with 3 successive portions of 50 ml of hot deionised water. The filter paper was washed with 3 successive portions of 50 ml of hot deionised water. The soil was scraped from the filter paper with a spatula and then the filter paper and the spatula were washed with a jet of hot deionised water letting the washing into the beaker. The sides of the beaker were washed down with deionised water and sufficient deionised water was added to give approximately 2 cm depth of suspension in the beaker. Then 10 ml of Calgon solution was pipetted into each beaker. The solution was dispersed for 5 minutes using the ultrasonic bath (Sonicor - Model No. : SC-120T).

25 ml of Calgon solution was pipetted into a weighed porcelain basin, evaporated to dryness on a steam bath and then dried in a 110 °C oven overnight and reweighed. This was carried out to find out what weight of Calgon was added to the suspension as its weight must be subtracted from the weights obtained for silt plus clay and clay.

2.10.2.2. FRACTIONATION OF COARSE AND FINE SAND

A 1 litre graduated cylinder was set up with a larger filter funnel in the neck. A 180 µm sieve and a 53 µm sieve were banked together and placed in the funnel with 180 µm sieve on the top. These sieve sizes allow for the separation of the coarse plus medium and the fine sand fractions. The soil suspension was poured into the 180 µm sieve. The sides of the beaker were washed using a wash bottle contained deionised water and rubber coated glass rod to ensure that all the soil was removed from the beaker. The soil was washed through 180 µm sieve until the coarse and medium sand appeared clean. The contents of the 53 µm sieve were washed in the same way. The sieves and funnel were removed and the volume of the cylinder was made up to 1 litre with deionised water. The contents of the sieves were washed into a weighed and labelled porcelain basins and evaporated to dryness on a steam bath. The basins were then transferred into a 110 °C oven and left overnight, cooled in a desiccator for 30

minutes and reweighed. The percent coarse sand plus medium sand and fine sand was then calculated on an oven dry basis in the soil mineral material.

2.10.2.3. FRACTIONATION OF THE SILT PLUS CLAY

The cylinders were kept at a constant room temperature. The temperature of the suspension was noted and appropriate time for the silt plus clay at a depth of 20 cm and a sampling depth of 10 cm for clay particles were selected from the table which was prepared by the Soil Survey of England and Wales (Hodgson, 1976).

Each cylinder was shaken thoroughly for one minute to ensure that all the soil was in suspension. The cylinder was then placed on the pipetting stand. Immediately a clean dry 50 ml pipette was lowered down into the cylinder with the tap closed, until it just failed to touch the surface of the liquid and the height on the scale was noted. About 20 seconds before the required time, the pipette was lowered down gently to exactly 20 cm depth. The tap was opened at the appropriate time and sample of slightly more than 50 ml of the suspension was taken. The tap was closed and the pipette was then removed from the cylinder and the volume was adjusted to 50 ml. The solution was run into a weighed and labelled basin. The shaking was then repeated to obtain a duplicate sample in the same way. The samples were evaporated to dryness on a steam bath and then dried at 110 °C in an oven overnight. The samples were then removed, cooled in a desiccator for 30 minutes and weighed.

2.10.2.4. FRACTIONATION OF THE CLAY

The cylinder was left undisturbed on a flat table for the appropriate time to allow the silt particles to settle down. The temperature of the suspension was noted and the sample of clay (25 ml suspension) was taken at 10 cm depth by similar procedure as described for silt plus clay except that the cylinder was not shaken between pipetting the samples. The solution was then run into a weighed and labelled porcelain basin, evaporated to dryness on a steam bath and then left in a 110 °C oven overnight. The samples were then removed, cooled in a desiccator for 30 minutes and weighed.

Textural class of soil was determined with the help of a triangular chart prepared by the Soil Survey of England and Wales (Hodgson, 1976).

2.11. DETERMINATION OF MACRONUTRIENT IONS

2.11.1. AUTOMATED DETERMINATION OF SOIL INORGANIC NITROGEN

The colorimetric method for the determination of inorganic forms of nitrogen (ammonium, nitrate and nitrite nitrogen) was used in the soil and water analysis.

Analysis was carried out using a Technicon Autoanalyzer II system comprising a sampler, proportioning pump, a manifold water bath at a constant temperature of 37 °C and colorimeter equipped with 650 nm filter and phototubes. Results of analysis were recorded with a single pen chart recorder. The system was connected to a BBC microcomputer which was used for the measurement of the peak heights and calculation of results. The reagent bottles were also put in a separate water bath at a constant temperature of 25 °C.

2.11.1.1. STANDARD METHOD FOR AMMONIUM NITROGEN DETERMINATION (BERTHELOT METHOD)

Ammonium is 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 is such that ammonium nitrogen can be determined in the range 0.0 to 1.0 mg /l and with care 0.0 to 0.1 mg /l (Brown, 1973). The schematic diagram of the flow system of the standard method is shown in figure 2.1.

REAGENTS :

Analar grade reagents and nitrogen free deionised water produced by Purite ***select*** (Purite Limited) were used throughout. These reagents were prepared in a fume cupboard. A fume cupboard was cleared and a 2 figure top pan balance was set up. Care was taken to ensure that the balance was properly locked before attempting to move it. The fan was switched off while actual weighing was in progress but otherwise was kept running. Any spills were cleared up immediately. When finished, the balance was wiped down and returned to the balance bench. Finally, the fume cupboard was wiped down.

(a)- Alkaline Phenol :

***** Phenol is highly toxic and caustic. Preparation of this reagent should be carried out in the fume cupboard. Ensure that you are familiar with the MSDS sheet and precautions for handling phenol. *****

22.5 g of sodium hydroxide was dissolved in approximately 900 ml deionised water in a 1 litre dark glass bottle and the solution was allowed to cool to room temperature. Working in a fume cupboard, 50 g phenol was weighed very carefully into a 1 litre beaker. Approximately 500 ml sodium hydroxide solution was added and the contents were stirred carefully with a glass rod to dissolve the phenol. Further sodium hydroxide solution was added if necessary. The solution was returned to the bottle and degassed in the ultrasonic bath for 10 minutes. The volume was made to 1 litre with degassed deionised water and the contents were mixed gently. A plastic stopper was used not a glass one.

(b)- Complexing Reagent :

***** Sodium nitroprusside is highly toxic. This reagent should be prepared in a fume cupboard. *****

50 g potassium sodium tartrate and 50 g sodium citrate were dissolved in approximately 900 ml deionised water in a 1 litre bottle and degassed for 10 minutes in the ultrasonic bath. 1.2 g sodium nitroprusside was weighed carefully into a 100 ml beaker, 50 ml degassed deionised water was added and the contents were stirred gently using a magnetic stirrer. The resulting solution was added to the citrate tartrate solution and degassed in the ultrasonic bath for 10 minutes. 1 ml of 15% Brij.-35 solution was added and the volume was made to 1 litre with degassed deionised water and the contents were mixed gently.

(c)- Sodium Hypochlorite Solution (Approximately 0.5 %) :

***** Sodium hypochlorite is a caustic bleaching agent. It will produce chlorine gas with acids. This reagent should be prepared in a fume cupboard. ******

Using a measuring cylinder 50 ml of sodium hypochlorite solution (12 % w/v available chlorine) was added to 1 litre degassed deionised water and the contents were mixed gently.

(d)- Ammonium nitrogen standard stock solution (1000 mg /l) :

Ammonium sulphate was dried at 105 °C for 1 hour and cooled in a desiccator for 30 minutes. 4.717 g of dry ammonium sulphate was dissolved in approximately 900 ml deionised water in 1 litre volumetric flask and the volume was made to 1 litre with deionised water. The solution was stored at 2 °C. Working standards were prepared by the dilution in the appropriate extracting solutions.

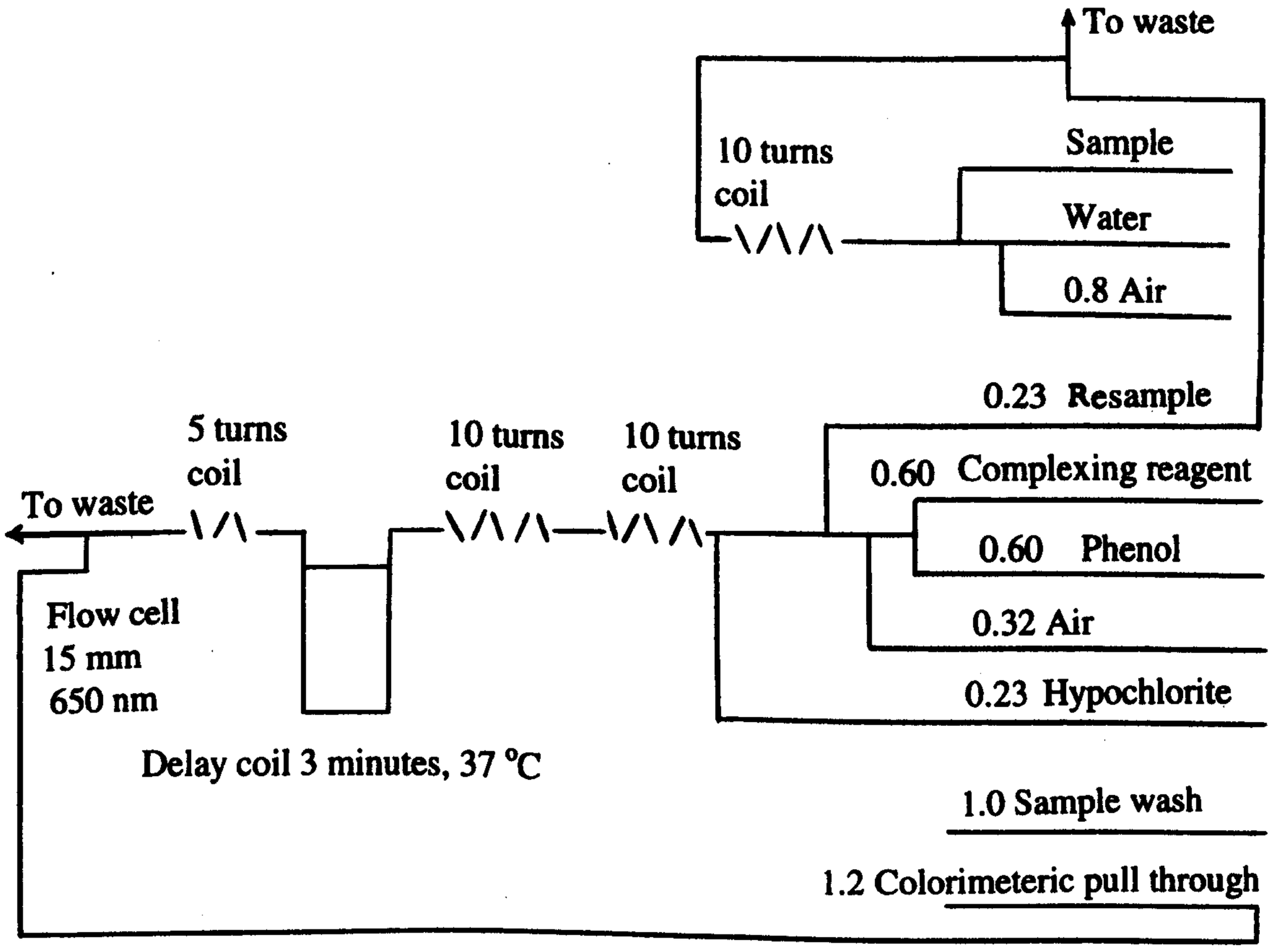


Figure 2.1. : Technicon Autoanalyzer II manifold for the determination of NH_4-N.

PROCEDURE :

The manifold (Figure 2.1.) can be used for the determination of ammonium in water, 2 M and 1 M potassium chloride, 0.5 M potassium sulphate and several other extractants. The flow rate of the standard method is shown in Table 2.1.

Table 2.1. : Reagent flow rate of the standard method for the ammonium-N determination.

Tube	Flow rate (ml /min)
Sample	0.23
Hypochlorite	0.23
Complexing reagent	0.60
Alkaline phenol	0.60
Air	0.32
Pull through	1.20

The 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 50 per hour but where dilution was required the sampling rate was reduced to 40 per hour. The colour was developed in the manifold water bath at 37 °C. The colour intensity was measured at 650 nm. The air was cleaned of atmospheric ammonia by bubbling through 5 % HCl solution. The calibration graph for ammonium is linear from 0.0 to 5 mg NH_4-N /l. Samples with ammonium - nitrogen concentrations higher than 5 mg/l were diluted into the range 0.0 to 5 mg/l using an inbuilt diluter.

The standard method for ammonium nitrogen determination was developed as described in section 3.1.2.1.

2.11.1.2. DETERMINATION OF AMMONIUM-N (TOTAL NITROGEN) IN KJELDAHL DIGESTS

The plant material was digested by the method of Bremner and Mulvaney (1982) as described in section 2.21. Ammonium nitrogen was determined in the

Kjeldahl digests by the Technicon Autoanalyzer II system as described in section 3.1.3.2. with certain modifications as follows :

ADDITIONAL REAGENTS :

In addition to the reagents used for the determination of ammonium as described in section 3.1.3.2., the following reagents were prepared :

(a)- Wash chamber solution (5 % H_2SO_4) :

Carefully 50 ml of concentrated sulphuric acid was dissolved in about 800 ml deionised water, then cooled and the volume was made to 1 litre with deionised water.

(b)- Neutralising solution :

3.6 g of NaOH was dissolved in a litre of deionised water.

PREPARATION OF THE WORKING STANDARD :

(a)- Ammonium nitrogen standard stock solution (1000 mg /l) :

Dry Ammonium sulphate was dried at 105 °C for 1 hour and cooled in a desiccator for 30 minutes. 4.717 g of dry ammonium sulphate was dissolved in approximately 900 ml deionised water in 1 litre volumetric flask and the volume was made to 1 litre with deionised water. The solution was stored at 2 °C. Working standards were prepared by the dilution in the appropriate extracting solutions.

(b)- Ammonium standard 0.0 mg /l :

5 ml of concentrated sulphuric acid and a half Kjeldahl tablet were added to a digestion tube. Then the tube was heated to dissolve the catalyst tablet and when cooled the contents were diluted carefully and transferred to a 100 ml volumetric flask. The volume was made to 100 ml with deionised water.

(c)- Ammonium standard 100 mg /l :

5 ml of concentrated sulphuric acid and a half Kjeldahl tablet were added to a digestion tube. Then the tube was heated to dissolve the catalyst tablet and when cooled the contents were diluted carefully and transferred to a 100 ml volumetric flask. 10 ml of 1000 mg /l stock ammonium standard solution was added and the volume was made up to 100 ml with deionised water.

PROCEDURE :

The filtered solutions were analysed directly on a Technicon Autoanalyzer II system. The samples were run at the rate of 40 per hour with a dilution / neutralisation step before the main manifold. The calibration graph for ammonium is linear from 0.0 to 100 mg NH_4-N /l.

Dilution / neutralisation step :

sample wash solution	5 % v/v H_2SO_4.
Dilution ratio	20 : 1
Neutralising solution	2.0 ml /minute.
Sample	0.1 ml /minute.
Air	0.8 ml /minute.

2.11.1.3. AUTOMATED DETERMINATION OF NITRATE AND NITRITE NITROGEN

In the automated system, nitrate nitrogen is quantitatively reduced to nitrite nitrogen followed by determination of the nitrite using the Greiss reagent. The method, therefore, measures nitrate plus nitrite. The nitrite nitrogen can be measured separately on the same manifold by omitting the reduction reagents (Best, 1976). The schematic diagram of the flow system of nitrate and nitrite is shown in Figure 2.2.

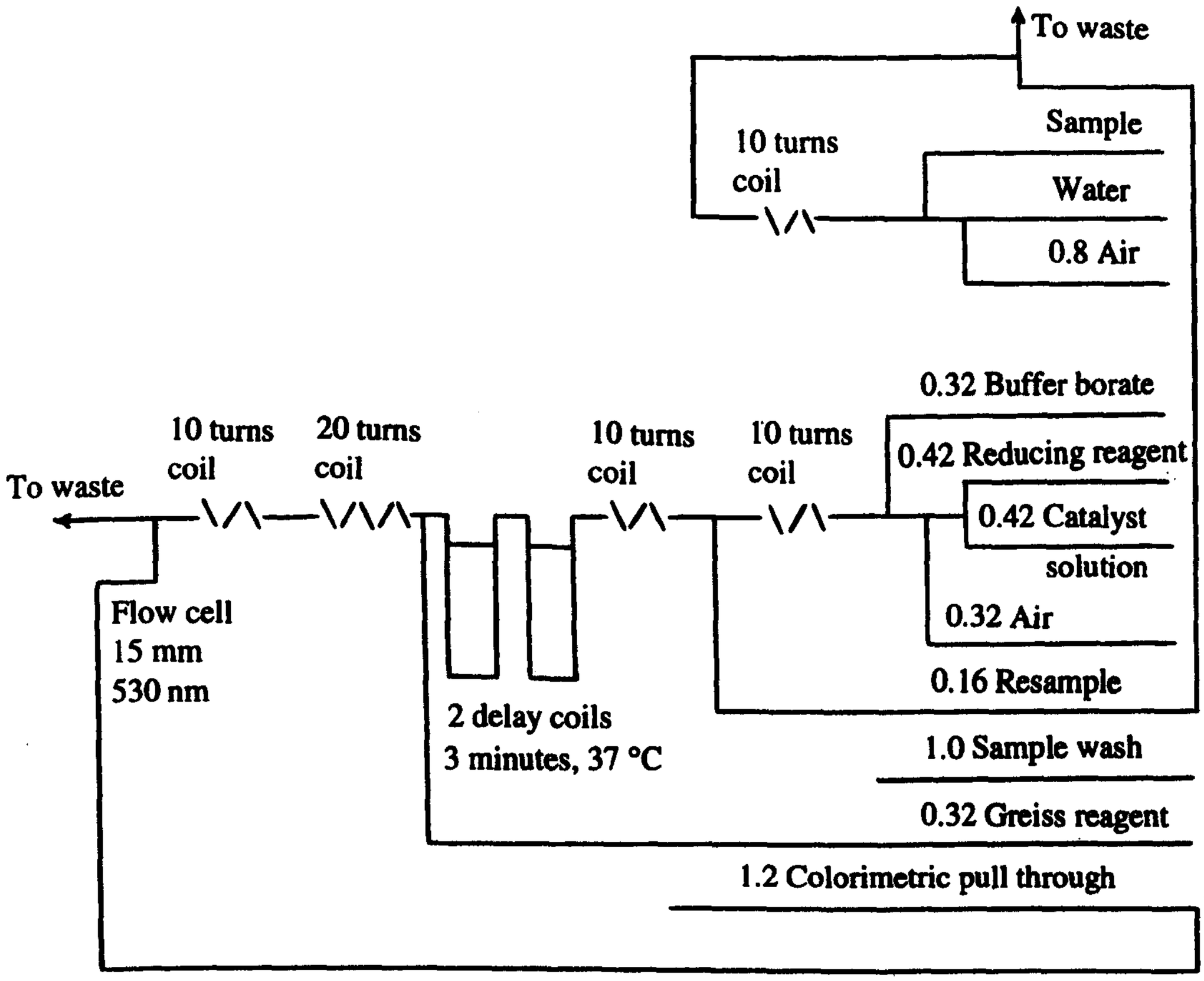


Figure 2.2. : Technicon Autoanalyzer II manifold for the determination of NO_3-N and NO_2-N.

REAGENTS :

Analar grade reagents and nitrogen free deionised water were used throughout. These reagents were prepared in a fume cupboard. A fume cupboard was cleared and a 2 figure top pan balance was set up. Care was taken to ensure that the balance was properly locked before attempting to move it. The fan was switched off while actual weighing was in progress but otherwise was kept running. Any spills were cleared up immediately. When finished, the balance was wiped down and returned to the balance bench. Finally, the fume cupboard was wiped down.

(a)- Buffer solution :

22.5 g sodium tetraborate and 2.5 g sodium hydroxide were dissolved in 900 ml deionised water and the volume was made to 1 litre with deionised water. The solution was degassed for 10 minutes in the ultrasonic bath.

(b)- Greiss reagent :

***** Concentrated hydrochloric acid is highly corrosive. Sulphanilamide is carcinogenic and a dust hazard. Prepare this reagent in a fume cupboard. *****

Carefully 100 ml concentrated hydrochloric acid was added (measuring cylinder) into approximately 800 ml deionised water. This solution was degassed for 10 minutes in the ultrasonic bath. Working in a fume cupboard 10.0 g sulphanilamide and 0.5 g N-1-naphthylene diamine dihydrochloride were weighed into a 1 litre beaker. 500 ml of the acid solution was added carefully and the contents were stirred gently using a magnetic stirrer. Return to the bottle, the volume was made to 1 litre with degassed deionised water and the contents were mixed gently. The solution was stored at 2 °C.

(c)- Reducing reagent :

***** Hydrazine sulphate is a dust hazard and a suspected carcinogen. Ensure that you are familiar with the MSDS sheet. Prepare this reagent in a fume cupboard. *****

0.30 g of hydrazine sulphate was weighed into a small beaker. The contents were transferred carefully to a 1 litre volumetric flask containing approximately 900 ml degassed deionised water. The volume was made up to the mark with deionised water without shaking and the hydrazine sulphate was dissolved with a magnetic stirrer keeping the top of the flask closed in order to prevent access of oxygen. 1 ml of 15 % Brij.-35 solution was added and the contents were mixed gently. If the hydrazine sulphate is shaken in a flask which is partly filled with deionised water, it will react with the oxygen content of the flask and decrease its reducing power.

(d)- Catalyst solution :

1 ml 2.47 % copper sulphate solution and 1 ml 15 % Brij.-35 solution were added to 1 litre of degassed deionised water and the contents were mixed gently.

(e)- Nitrate nitrogen standard stock solution (1000 mg /l) :

Sodium nitrate or potassium nitrate was dried at 105 °C for 1 hour and cooled in a desiccator for 30 minutes. 6.068 g of dried sodium nitrate or 7.218 g of dried potassium nitrate was dissolved in deionised water in 1 litre volumetric flask and the volume was made to 1 litre with deionised water. The solution was stored at 2 °C. Working standards were prepared by the dilution in the appropriate extracting solutions.

(f)- Nitrite nitrogen standard stock solution (1000 mg /l) :

Sodium nitrite was dried at 105 °C for 1 hour and cooled in a desiccator for 30 minutes. 4.926 g of dried sodium nitrite was dissolved in deionised water in 1 litre volumetric flask and the volume was made to 1 litre with deionised water. The solution was stored at 2 °C. Working standards were prepared by the dilution in the appropriate extracting solutions.

PROCEDURE :

The manifold (Figure 2.2.) can be used for the determination of nitrate and nitrite nitrogen in water and a range of extracting solutions.

Table 2.2. : Reagent flow rate of the standard method for the nitrate and nitrite nitrogen determination.

Tube	Flow rate (ml /min)
Sample	0.16
Reducing reagent	0.42
Catalyst solution	0.42
Buffer borate	0.32
Greiss reagent	0.32
Air	0.32
Pull through	1.20

The solutions were analysed for nitrate and nitrite using the manifold shown in Figure 2.2. along with standard solutions, blanks and zeros. The samples were run at the rate of 50 per hour but where dilution was required the sampling rate was reduced to 40 per hour. The nitrate was reduced to nitrite by adding copper sulphate and hydrazine sulphate solutions to the sample as it passed through the water bath set at 37 °C. The nitrite - nitrogen was determined by a diazotization coupling reaction whereby a pink colour was formed. The colour intensity was measured at 530 nm. Nitrate has a linear calibration graph in the range 0.0 to 2.0 and curved calibration graph in the range 2.0 to 5.0 mg /l NO_3-N. The calibration graph for nitrite is linear from 0.0 to 4.0 mg /l NO_2-N. Samples with nitrate - nitrogen concentrations higher than 5.0 mg /l were diluted into the range 0.0 to 5.0 mg/l using an inbuilt diluter.

For the determination of nitrite nitrogen, the reducing reagents were replaced with nitrogen free deionised water containing 1 ml per litre of 15 % Brij.-35 solution.

2.11.2. AUTOMATED DETERMINATION OF SOIL INORGANIC PHOSPHORUS

Technicon Autoanalyzer II was used for the determination of soil inorganic phosphorus. The method is based on the formation of a phosphomolybdate complex using antimony to accelerate the formation of the faintly yellow coloured product. The coloured product is then reduced using ascorbic acid to give a more intense blue colour which may be measured at 880 or 660 nm. The method is applicable to water samples and a wide range of soil extract solutions and digests of plant or soil material.

REAGENTS :

Analar grade reagents and phosphorus free deionised water were used throughout. These reagents were prepared in a fume cupboard. A fume cupboard was cleared and a 2 figure top pan balance was set up. Care was taken to ensure that the balance was properly locked before attempting to move it. The fan was switched off while actual weighing was in progress but otherwise was kept running. Any spills were cleared up immediately. When finished, the balance was wiped down and returned to the balance bench. Finally, the fume cupboard was wiped down.

(a)- Acid molybdate solution :

***** Ammonium molybdate and antimony potassium tartrate are toxic, concentrated sulphuric acid is highly toxic and corrosive and can react violently with water. This reagent should be prepared in a fume cupboard. *****

Carefully 60 ml concentrated sulphuric acid was added to approximately 700 ml deionised water in a 1 litre brown glass bottle and then the solution was cooled to room temperature. 5.2 g ammonium molybdate was added and dissolved by stirring. 0.1 g antimony potassium tartrate was dissolved in approximately 50 ml deionised water in a small beaker and this solution was added to the acid solution with mixing to avoid the formation of a precipitate. The reagent was degassed for 10 minutes in the ultrasonic bath and the volume was made up to 1 litre with degassed deionised water and the contents were mixed gently. This reagent is stable for at least 1 month if stored in the dark and can be degassed at intervals if required.

(b)- Ascorbic acid :

0.75 g ascorbic acid was dissolved in 100 ml degassed deionised water and dissolved by stirring gently on a magnetic stirrer. This reagent is unstable and must be prepared in the day of use.

(c)- Dilution water :

0.0 to 1.0 mg P /l range :

2 ml of aerosol - 22 wetting agent was added to 1 litre degassed deionised water and the contents were mixed gently.

0.0 to 5.0 mg P /l range :

1 ml of aerosol - 22 wetting agent was added to 1 litre degassed deionised water and the contents were mixed gently.

(d)- Phosphate-P standard stock solution (1000 mg /l) :

Potassium dihydrogen phosphate was dried at 105 °C for 1 hour and cooled in a desiccator for 30 minutes. 4.394 g of dry potassium dihydrogen phosphate was dissolved in approximately 900 ml deionised water in 1 litre volumetric flask and the volume was made to 1 litre with deionised water. The solution was stored at 2 °C. Working standards were prepared by the dilution in the appropriate extracting solutions.

PROCEDURE :

The manifold (Figure 2.3.) can be used for the determination of phosphate in water, acetic acid, acetate buffers, and several other extractants. For phosphorus determination in sodium bicarbonate or ammonium fluoride a separate manifold is required.

The solutions were analysed for phosphate using the manifold shown in Figure 2.3. along with standard solutions, blanks and zeros. The samples were run at the rate of 50 per hour but where dilution was required the sampling rate was reduced to 40 per hour. The colour was developed in the water bath at 37 °C. The colour intensity was measured at 880 nm. Phosphate has a linear calibration graph in the range 0.0 to 5.0 mg PO_4 - P /l. Samples with phosphate concentrations higher than 5 mg /l were diluted into the range 0.0 to 5 mg /l using an inbuilt diluter.

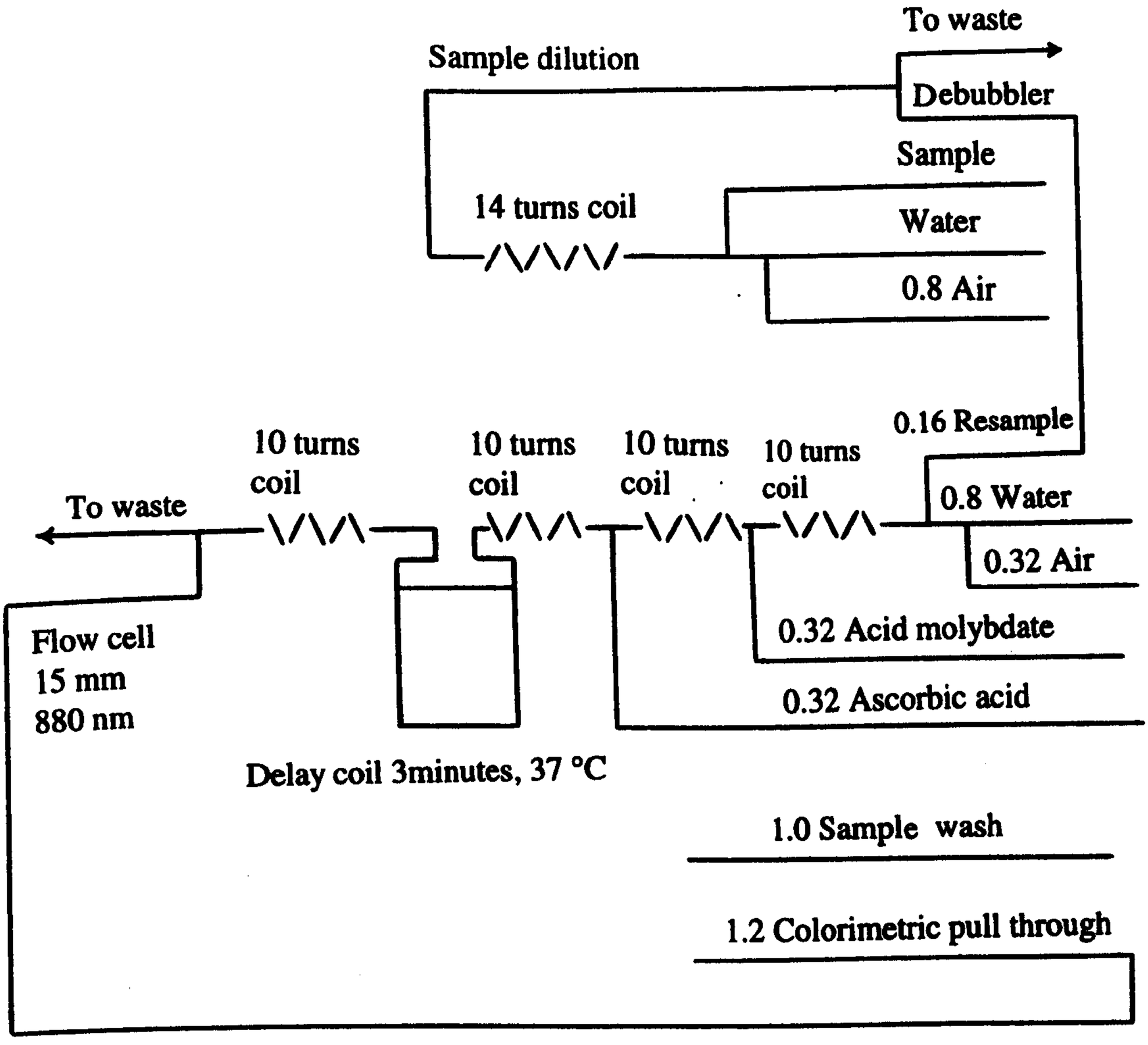


Figure 2.3. : Technicon Autoanalyzer II manifold for the determination of phosphate - P.

Manifold configuration for water samples :

Sample (ml /min)	Diluent water (ml /min)	Max sample P (mg /l)
0.16	0.80	5.0
0.42	0.60	2.0
0.60	0.42	1.0

BACKGROUND COLOUR CORRECTION :

60 ml concentrated sulphuric acid was added carefully to approximately 700 ml deionised water in a 1 litre glass bottle and then the solution was cooled to room temperature. 1.67 g ammonium sulphate was added and dissolved by stirring. The reagent was degassed for 10 minutes in the ultrasonic bath and the volume was made up to 1 litre with degassed deionised water and the contents were mixed gently. This reagent is stable for several months and can be degassed at intervals if required.

The acid molybdate solution was replaced with this reagent and the samples were returned at the sample colorimeter settings.

2.11.3. DETERMINATION OF POTASSIUM IN SOIL AND WATER

Potassium was determined by the Flame photometer (Corning - Model : 410).

2.11.3.1. REAGENTS

Potassium standard stock solution (1000 mg /l) :

This is a Flame photometer standard stock solution (1000 mg K^+ /l). It is manufactured by Ciba Corning Diagnostics Ltd.

Working standard solutions and concentrations :

Potassium working standard solutions were prepared by dilution of 1000 mg K^+ /l standard stock solution with appropriate extracting solutions.

2.11.3.2. PROCEDURE

The Flame photometer was calibrated using 0.0, 10, 20, 30, 40 and 50 mg K^+ /l working standard solutions in the case of the soil samples and 0.0 to 5.0 mg K^+ /l working standard solutions in the case of the water samples. The calibration graph is curved for 0.0, 10, 20, 30, 40 and 50 mg K^+ /l working standard solutions and is linear for 0.0 to 5.0 mg K^+ /l working standard solutions. After getting a stable readings for the

working standard solutions, the samples were run and their stable readings were recorded. The calculations were made by a calibration curve program on a BBC microcomputer.

2.11.4. DETERMINATION OF SODIUM IN WATER

Sodium was determined by the Flame photometer.

2.11.4.1. REAGENTS

Sodium standard stock solution (1000 mg /l) :

This is a Flame photometer standard stock solution (1000 mg Na^+ /l). It is manufactured by Ciba Corning Diagnostics Ltd.

Working standard solutions and concentrations :

Sodium working standard solutions were prepared by dilution of 1000 mg Na^+ /l standard stock solution with appropriate extracting solutions.

2.11.4.2. PROCEDURE

The Flame photometer was calibrated using 0.0 to 2.5 mg Na^+ /l working standard solutions. The calibration graph is linear for 0.0 to 2.5 mg Na^+ /l working standard solutions. After getting a stable readings for the working standard solutions, the samples were run and their stable readings were recorded.

2.11.5. DETERMINATION OF CALCIUM IN SOIL AND WATER

Calcium was determined by the Atomic Absorption Spectrophotometer (Perkin Elmer - Model : 1100 B).

2.11.5.1. REAGENTS

Calcium standard stock solution (1000 mg /l) :

This is a calcium nitrate standard stock solution (1000 mg Ca /l). It was supplied by BDH Spectrosol.

Releasing agent :

$SrCl_2$ solution was used to release calcium from the interference with phosphate, silicate and sulphate.

A 10 % $SrCl_2$ solution or 30 % $SrCl_2.6H_2O$ solution was used as a releasing agent for the determination of calcium in the soil samples. A 1.0 % $SrCl_2$ solution or 3.0 % $SrCl_2.6H_2O$ solution was used as a releasing agent for the determination of calcium in the water samples. This was added to standards and samples.

Working standard solutions and concentrations :

Calcium working standard solutions were prepared by dilution of 1000 mg Ca /l standard stock solution with appropriate extracting solutions and with the addition of releasing agent.

2.11.5.2. PROCEDURE

The Atomic Absorption Spectrophotometer was calibrated using 0.0 to 5.0 mg Ca /l working standard solutions. The calibration graph is linear for 0.0 to 5.0 mg Ca /l working standard solutions. After getting a stable readings for the working standard solutions, the samples were run and their readings were recorded.

2.11.6. DETERMINATION OF MAGNESIUM IN SOIL AND WATER

Magnesium was determined by the Atomic Absorption Spectrophotometer.

2.11.6.1. REAGENTS

Magnesium standard stock solution (1000 mg /l) :

This is a magnesium nitrate standard stock solution (1000 mg Mg /l). It was supplied by BDH Spectrosol.

Releasing agent :

$SrCl_2$ solution was used to release magnesium from the interference with phosphate, silicate and sulphate.

A 10 % $SrCl_2$ solution or 30 % $SrCl_2.6H_2O$ solution was used as a releasing agent for the determination of magnesium in the soil samples. A 1.0 % $SrCl_2$ solution or 3.0 % $SrCl_2.6H_2O$ solution was used as a releasing agent for the determination of magnesium in the water samples. This was added to standards and samples.

Working standard solutions and concentrations :

Magnesium working standard solutions were prepared by dilution of 1000 mg Mg /l standard stock solution with appropriate extracting solutions and with the addition of releasing agent.

2.11.6.2. PROCEDURE

The Atomic Absorption Spectrophotometer was calibrated using 0.0 to 0.5 mg Mg /l working standard solutions. The calibration graph is linear for 0.0 to 0.5 mg Mg /l working standard solutions. After getting a stable readings for the working standard solutions, the samples were run and their readings were recorded.

2.12. DETERMINATION OF TOTAL METALS BY THE ATOMIC ABSORPTION SPECTROPHOTOMETER

2.12.1. DETERMINATION OF IRON IN WATER

Iron was determined by the Atomic Absorption Spectrophotometer.

2.12.1.1. REAGENTS

Iron standard stock solution (1000 mg /l) :

This is an iron (III) nitrate standard stock solution (1000 mg Fe /l). It was supplied by BDH Spectrosol.

Working standard solutions and concentrations :

Iron working standard solutions were prepared by dilution of 1000 mg Fe /l standard stock solution with appropriate extracting solutions.

2.12.1.2. PROCEDURE

The Atomic Absorption Spectrophotometer was calibrated using 0.0 to 5.0 mg Fe /l working standard solutions. The calibration graph is linear for 0.0 to 5.0 mg Fe /l working standard solutions. After getting a stable readings for the working standard solutions, the samples were run and their readings were recorded.

2.12.2. DETERMINATION OF COPPER IN SOIL

Copper was determined by the Atomic Absorption Spectrophotometer.

2.12.2.1. REAGENTS

Copper standard stock solution (1000 mg /l) :

This is copper (II) nitrate [$Cu(NO_3)_2$] standard stock solution (1000 mg Cu /l). It was supplied by BDH Spectrosol.

Working standard solutions and concentrations :

Copper working standard solutions were prepared by dilution of 1000 mg Cu /l standard stock solution with appropriate extracting solutions.

2.12.2.2. PROCEDURE

The Atomic Absorption Spectrophotometer was calibrated using 0.0 to 5.0 mg Cu /l working standard solutions. The calibration graph is linear for 0.0 to 5.0 mg Cu /l working standard solutions. After getting a stable readings for the working standard solutions, the samples were run and their readings were recorded.

2.12.3. DETERMINATION OF NICKEL IN SOIL

Nickel was determined by the Atomic Absorption Spectrophotometer.

2.12.3.1. REAGENTS

Nickel standard stock solution (1000 mg /l) :

This is nickel nitrate [$Ni(NO_3)_2.6H_2O$] standard stock solution (1000 mg Ni /l). It was supplied by BDH Spectrosol.

Working standard solutions and concentrations :

Nickel working standard solutions were prepared by dilution of 1000 mg Ni /l standard stock solution with appropriate extracting solutions.

2.12.3.2. PROCEDURE

The Atomic Absorption Spectrophotometer was calibrated using 0.0 to 2.0 mg Ni /l working standard solutions. The calibration graph is linear for 0.0 to 2.0 mg Ni /l working standard solutions. After getting a stable readings for the working standard solutions, the samples were run and their readings were recorded

2.12.4. DETERMINATION OF ZINC IN SOIL

Zinc was determined by the Atomic Absorption Spectrophotometer.

2.12.4.1. REAGENTS

Zinc standard stock solution (1000 mg /l) :

This is zinc nitrate [$Zn(NO_3)_2.4H_2O$] standard stock solution (1000 mg Zn /l). It was supplied by BDH Spectrosol.

Working standard solutions and concentrations :

Zinc working standard solutions were prepared by dilution of 1000 mg Zn /l standard stock solution with appropriate extracting solutions.

2.12.4.2. PROCEDURE

The Atomic Absorption Spectrophotometer was calibrated using 0.0 to 1.0 mg Zn /l working standard solutions. The calibration graph is linear for 0.0 to 1.0 mg Zn /l working standard solutions. After getting a stable readings for the working standard solutions, the samples were run and their readings were recorded

2.12.5. DETERMINATION OF CHROMIUM IN SOIL

Chromium was determined by the Atomic Absorption Spectrophotometer.

2.12.5.1. REAGENTS

Chromium standard stock solution (1000 mg /l) :

This is chromium (III) nitrate standard stock solution (1000 mg Cr /l). It was supplied by BDH Spectrosol.

Working standard solutions and concentrations :

Chromium working standard solutions were prepared by dilution of 1000 mg Cr /l standard stock solution with appropriate extracting solutions.

2.12.5.2. PROCEDURE

The Atomic Absorption Spectrophotometer was calibrated using 0.0 to 5.0 mg Cr /l working standard solutions. The calibration graph is linear for 0.0 to 5.0 mg Cr /l working standard solutions. After getting a stable readings for the working standard solutions, the samples were run and their readings were recorded

2.12.6. DETERMINATION OF CADMIUM IN SOIL

Cadmium was determined by the Atomic Absorption Spectrophotometer.

2.12.6.1. REAGENTS

Chromium standard stock solution (1000 mg /l) :

This is cadmium nitrate [$Cd(NO_3)_2.4H_2O$] standard stock solution (1000 mg Cd /l). It was supplied by BDH Spectrosol.

Working standard solutions and concentrations :

Cadmium working standard solutions were prepared by dilution of 1000 mg Cd /l standard stock solution with appropriate extracting solutions.

2.12.6.2. PROCEDURE

The Atomic Absorption Spectrophotometer was calibrated using 0.0 to 2.0 mg Cd /l working standard solutions. The calibration graph is linear for 0.0 to 2.0 mg Cd /l working standard solutions. After getting a stable readings for the working standard solutions, the samples were run and their readings were recorded

2.12.7. DETERMINATION OF LEAD IN SOIL

Lead was determined by the Atomic Absorption Spectrophotometer.

2.12.7.1. REAGENTS

Lead standard stock solution (1000 mg /l) :

This is lead nitrate [$Pb(NO_3)_2$] standard stock solution (1000 mg Pb /l). It was supplied by BDH Spectrosol.

Working standard solutions and concentrations :

Lead working standard solutions were prepared by dilution of 1000 mg Pb /l standard stock solution with appropriate extracting solutions.

2.12.7.2. PROCEDURE

The Atomic Absorption Spectrophotometer was calibrated using 0.0 to 20 mg Pb /l working standard solutions. The calibration graph is linear for 0.0 to 20 mg Pb /l working standard solutions. After getting a stable readings for the working standard solutions, the samples were run and their readings were recorded

2.13. FLAME CONDITIONS OF ANALYSIS BY THE ATOMIC ABSORPTION SPECTROPHOTOMETER

Table 2.3. : Flame conditions of analysis of calcium, magnesium and iron.

Flame conditions	Ca	Mg	Fe
Wavelength (nm)	324.8	285.2	248.3
Lamp current	10.0	6.0	30.0
Fuel Flow (C_2H_2)	2.5	2.5	2.5
Air flow	8.0	8.0	8.0
Background correction	Off	On	On

Table 2.4. : Flame conditions of analysis of cadmium, chromium, copper, nickel, zinc and lead.

Flame conditions	Cd	Cr	Cu	Ni	Zn	Pb
Wavelength (nm)	228.8	357.9	324.8	232.0	213.9	283.3
Lamp current	8.0	12.0	10.0	30.0	10.0	15.0
Fuel Flow (C_2H_2)	2.5	3.5	2.5	2.5	2.5	2.5
Air flow	8.0	8.0	8.0	8.0	8.0	8.0
Background correction	On	Off	Off	On	On	On

2.14. ANALYSIS OF METALS BY THE INDUCTIVELY COUPLED PLASMA SPECTROSCOPY

Chromium, cadmium, nickel, zinc, lead, copper, boron, barium, manganese, magnesium and potassium were determined by the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) using a Thermounicam IRIS Radial ICP/CID. The analysis preferences are presented in Table 2.5. The plasma conditions are shown in Table 2.6. The analysis conditions of chromium, cadmium, nickel, zinc, lead, copper, boron, barium, manganese, magnesium and potassium are given in Table 2.7.

Table 2.5. : The analysis preferences.

Analysis preferences	
Number of repeats	3.0
Delay time (sec)	0.0
Sample flush time (sec)	60.0
Integration time :	
Low wavelength range (sec)	30.0
High wavelength range (sec)	5.0

Table 2.6. : The plasma conditions.

Plasma Conditions	
Auxiliary gas	Low
RF power	1150
Nebulizer pressure (PSI)	32
Flush pump rate (rpm)	100
Analysis pump rate (rpm)	100
Relaxation time (sec)	0.0

Table 2.7. : The analysis conditions of chromium, cadmium, nickel, zinc, lead, copper, boron, barium, manganese, magnesium and potassium.

Analysis conditions	Cd	Cr	Ni	Zn	Pb	Cu
Wavelength (nm) (order)	226.502 (116)	283.563 (93)	231.604 (114)	213.856 (123)	220.353 (119)	327.396 (80)
Subarray information :						
Subarray width	15	15	15	15	15	15
Subarray height	3	3	3	3	3	3
Centre width	1	2	1	1	2	1
Background position :						
Left position*	1	1	1	1	1	1
Right position*	12	15	15	15	15	15
Standard concentration	10 mg /l All	10 mg /l All	10 mg /l All	10 mg /l All	10 mg /l All	10 mg /l All
QC Check	5.0 mg /l All	5.0 mg /l All	5.0 mg /l All	5.0 mg /l All	5.0 mg /l All	5.0 mg /l All

* All background width = 1.

Table 2.7. : Continued.

Analysis conditions	B	Ba	Mn	Mg	K
Wavelength (nm) (order)	249.773 (105)	233.527 (113)	259.373 (101)	285.213 (92)	769.896 (34)
Subarray information :					
	15	15	15	15	15
Subarray width	3	3	3	3	3
Subarray height	1	2	2	2	1
Centre width					
Background position :					
	1	1	1	1	5
Left position*	15	13	15	15	15
Right position*					
Standard concentration	10 mg /l All	10 mg /l All	10 mg /l All	10 mg /l All	10 mg /l All
QC Check	5.0 mg /l All	5.0 mg /l All	5.0 mg /l All	5.0 mg /l All	5.0 mg /l All

2.15. DETERMINATION OF ANIONS BY ION CHROMATOGRAPHY

Fluoride, nitrite, nitrate, chloride, bromide, sulphate and phosphate were determined in the filtrate by Ion Chromatography (Model : DIONEX DX 500 Ion Chromatography System).

The system consisted of :

1- GP40 Gradient Pump.

2- LC10 Chromatography Module (AG11 Guard Column and AS 11 Separator Column and ASRS Anion Self Regenerating Suppresser).

3- ED40 Electrochemical Detector.

4- Computer running Peak Net Chromatography Software (version 4.3).

METHOD OF SEPARATION :

The seven anions were separated by gradient separation. The gradient separation is shown in Table 2.8.

Table 2.8. : The gradient separation.

Time (minutes)	Water	200 mM NaOH
0.0	97.5 %	2.5 %
1.0	97.5 %	2.5 %
10.0	85.0 %	15.0 %
10.1	97.5 %	2.5 %
15.0	97.5 %	2.5 %

METHOD PARAMETERS :

Eluent flow rate = 2.0 ml /min.

Injection loop = 25.0 µl.

Detector Mode = Conductivity.

SRS current = 300 mA.

The retention time and standard calibration details are shown in Table 2.9.

Table 2.9. : The retention time and standard calibration details.

Component	Retention time	Standard concentration
Fluoride	0.96	1.0 mg /l
Chloride	1.70	1.0 mg /l
Nitrite	2.01	1.0 mg /l
Bromide	3.23	1.0 mg /l
Nitrate	3.36	1.0 mg /l
Sulphate	4.62	1.0 mg /l
Phosphate	7.72	1.0 mg /l

2.16. DETERMINATION OF CATION EXCHANGE CAPACITY IN SOIL

Negative charges on the clay and organic matter in soils provide sites at which cations can be held by ion exchange. Nutrients held in this way are considered to be available to plants. Thus a measure of both the total amount of negative charge and the percentage holding nutrient cations will give information regarding the potential and actual fertility of a soil.

The measurement of cation exchange capacity gives the total quantity of negative charge in a soil, and so indicates the soil's ability to hold cations. The cation exchange capacity is made up of :

(a)- Fixed charge on the clays to imperfections in the crystal lattice (isomorphous substitution).

(b)- pH dependent charges on clay edges and organic matter.

Therefore, it is important to state at what pH the cation exchange capacity is measured. The method described below involves saturation of the negative sites with K^+ ions at pH 7.0. As other methods use different saturating (or index) cations, it is also important to state which index ion is being used.

The negative charges were measured with K^+ ions by leaching the soil with an excess of potassium, so removing other exchangeable cations by mass action. The excess K^+ ions were then removed with 95 % ethanol and K^+ hold on the negative sites were displaced by leaching the soil with NH_4^+ ions. The solution containing the displaced K^+ ions was then made to a known volume and the concentration of K^+ was determined by flame emission using a Flame photometer.

2.16.1. PROCEDURE

Triplicate samples of 10 g air-dried less than 2 mm sieved soil were weighed out accurately in 100 ml beaker. The soil was mixed thoroughly with 2 volumes of acid-washed sand to assist percolation. A glass column with small pieces of glass wool were plugged in the bottom, 1 cm depth of acid-washed sand was placed on the top of the glass wool and the soil/sand mixtures was placed on the top of the acid-washed sand in

the columns. Then another half cm depth of acid-washed sand was placed on the top of the soil/sand mixtures.

Deionised water was poured in the column to get a saturated soil and then the leachate of deionised water was discarded. Each column was leached with 200 ml of 1M potassium acetate at pH 7.0 and then the leachate was discarded. Excess potassium acetate was removed by leaching each column with 200 ml 95 % ethanol and then the leachate was discarded. The column was washed from outside by deionised water , then wiped by a tissue paper to remove any spillage of potassium acetate.

Each column was leached with 200 ml of 1 M ammonium acetate at pH 7.0 and the leachate was collected in 250 ml volumetric flask. When all the ammonium acetate had leached through the column the flask was made up to the mark with deionised water and mixed well.

Dilution of this solution was made by a factor of 1 : 100. Potassium was determined in the diluted leachate by the Flame photometer as described in section 2.11.3. using a standard series 0.0, 10, 20, 30, 40 and 50 mg K^+ /l in 0.01 M ammonium acetate solution. If necessary, the samples were diluted again as appropriate to fit in the calibration graph and the ammonium acetate solution was maintained at 0.2 M.

If the analysis can not carried out immediately, the solution was stored in polythene bottles in order to avoid contamination of potassium from the glass.

*** Notes :**

1- When running this column, do not allow the soil to stand in potassium acetate over a long period.

2- Never allow the column to run dry, except at the end of the ammonium acetate leach.

2.16.2. CALCULATION OF CATION EXCHANGE CAPACITY

The concentration of potassium in solution was measured in mg /l. Cation exchange capacity was quoted in units of c $mole_c$ /kg. This was because the concentration of a cation was being used as a measure of negative charge :

Using the measured potassium concentration in mg /l, cation exchange capacity was calculated as follows :

Weight of K^+ extracted (mg) =

Concentration of K^+ (mg /l) × Dilution factor × Volume of leachate (l)

c $mole_c$ of K^+ /kg soil =

$$\frac{\text{Weight of } K^+ \text{ extracted (mg)}}{\text{Atomic weight of potassium} \times 10 \times \text{weight of oven dried soil (kg)}}$$

Where :

Atomic weight of potassium = 39.1

c $mole_c$ /kg is equivalent to meq. /100g.

2.17. EXTRACTION OF INORGANIC NITROGEN FROM SOIL

2.17.1. REAGENTS

PREPARATION OF 0.5 M POTASSIUM SULPHATE SOLUTION :

This solution was prepared according to Khan (1987) as follows :

Analar grade potassium sulphate reagent was used. 87.12 g potassium sulphate was dissolved in about 800 ml deionised water in 1.5 litre beaker and the volume was made up to 1 litre with deionised water. The solution was purified of ammonium nitrogen contamination by first raising the pH to 11.0 with 1 M potassium hydroxide. It was then boiled and stirred for a period of 15 minutes to give off the ammonia gas. The solution was allowed to cool and the pH was readjusted to pH 6.0 with 0.5 M sulphuric acid. Then the contents were transferred to a 1 litre volumetric flask with deionised water and the volume was made up to the mark with deionised water.

As there is no simple method for removal of nitrate nitrogen from the extracting solution, the potassium sulphate was first tested and a batch number with a low level of nitrate impurities was selected for use.

2.17.2. EXTRACTION PROCEDURE

2.5 g fresh soil passed through a 2 mm sieve was weighed in duplicate, into a 100 ml glass bottle. 50 ml of 0.5 M K_2SO_4 solution was added. Then the bottle was shaken for 2 hours in a shaker in the cold room at 2 °C. The shaken samples were filtered through Whatman No. 2 filter paper [filter paper pre washed with 0.5 M H_2SO_4 solution according to Shah method (1988) as described in section 2.2.] and the filtrate was collected in a 100 ml plastic bottle. NH_4-N, NO_3-N and NO_2-N were determined in the filtrate. The blank determination was carried out without soil.

2.18. EXTRACTION OF PHOSPHATE FROM SOIL

The extraction of phosphorus from soil was carried out according to MAFF /ADAS method No. 59 (1986).

2.18.1. REAGENTS

PREPARATION OF 0.5 M SODIUM BICARBONATE SOLUTION :

210 g $NaHCO_3$ was dissolved with deionised water in a 400 ml beaker. Then the contents were transferred with deionised water to a 5 litres conical flask. 25 ml of 0.05 % m/v polyacrylamide solution was added. The volume was made up to the mark with deionised water. Then drops of 3 M NaOH solution was added until the pH, measured with a pH meter (Mettler - Model : Delta 320), is 8.5 at 20 °C. Before use the pH meter was standardised with the pH 7.0 and pH 4.0 buffer solutions instead of buffer solution of pH 8.5. The pH of the reagent was checked on the day of use.

PREPARATION OF POLYACRYLAMIDE SOLUTION, 0.05 % m/v :

0.5 g polyacrylamide was dissolved in approximately 600 ml of deionised water by stirring for several hours. The contents were left overnight to make sure it is dissolved. When the polymer was dissolved, the contents were diluted to 1 litre with deionised water.

PREPARATION OF 2.5 N H_2SO_4 SOLUTION :

69.0 ml of concentrated H_2SO_4 was diluted in approximately 800 ml deionised water in a 1 litre volumetric flask. Then the volume was made up to the mark with deionised water.

2.18.2. EXTRACTION PROCEDURE

2.5 g air dried soil passed through a 2 mm sieve was weighed, in duplicate, into a 100 ml glass bottle. 50 ml 0.5 M $NaHCO_3$ solution was added. The bottle was shaken in the end - over - end shaker at 20 °C for 30 minutes. The shaken samples were filtered through a Whatman No. 2 filter paper and the filtrate was collected in a 100 ml plastic bottle. Phosphorus was measured in the filtrate. The blank determination was carried out without soil.

2.18.3. NEUTRALISATION PROCEDURE

Sodium bicarbonate soil extracts were neutralised as follows :

5.0 ml soil extract was placed into a 15 ml glass bottle. 2.0 ml of 2.5 N H_2SO_4 solution was added gradually. Then the glass bottle was closed by a plastic stopper and the contents were shaken well. The stopper was taken away and the glass bottle was left for 30 minutes for neutralisation and evolution of CO_2 and then the contents were shaken well again to make sure that all CO_2 gas disappeared. Phosphorus was determined in the neutralised extracts.

2.19. EXTRACTION OF POTASSIUM AND MAGNESIUM FROM SOIL

The extraction of potassium and magnesium from soil was carried out according to MAFF /ADAS methods No. 40 and 63 (1986).

2.19.1. REAGENTS

PREPARATION OF 1M NH_4NO3 SOLUTION :

80.0 g ammonium nitrate was dissolved with deionised water in a 600 ml beaker. Then the contents were transferred with deionised water to a 1 litre volumetric flask and the volume was made up to the mark with deionised water.

2.19.2. EXTRACTION PROCEDURE

5.0 g air dried soil passed through a 2 mm sieve was weighed, in duplicate, into a 100 ml glass bottle. 50 ml of 1 M NH_4NO_3 solution was added. The bottle was shaken for 30 minutes in the end - over - end shaker. The shaken samples were filtered through a Whatman No. 1 filter paper and the filtrate was collected in a 100 ml plastic bottle. Potassium was measured in the filtrate by the Flame photometer as described in section 2.11.3. Magnesium was measured in the filtrate by the Atomic Absorption Spectrophotometer as described in section 2.11.6. The blank determination was carried out without soil.

2.20. ACID DIGESTION OF SOIL

Digestion of soil was done using a 3 : 1 Hydrochloric acid / Nitric acid mixture known as Aqua Regia.

APPARATUS :

Tecator 40 tube block digester and Technicon Autoanalyzer system.

PREPARATION OF AQUA REGIA SOLUTION :

The solution contains three parts 6 M HCl to one part 69 % HNO_3 as follows :

300 ml of concentrated HCl + 200 ml of concentrated HNO_3 + 300 ml deionised water.

Where :

The concentration of the concentrated HCl is 12 M.

DIGESTION PROCEDURE :

2.5 g ground soil was weighed exactly using a four figure balance, in duplicate. Each sample was placed in a block digestion tube and 10 ml of Aqua Regia solution was added. The tubes were allowed to stand for at least twelve hours to allow the acid to equilibrate with the soil. The tubes were then placed in the digestion block and the temperature was set at 125 °C. The extraction unit was switched on to remove the brown NO_2 gas evolved during the digestion. The digestion block was run for at least 3 hours, until the tubes were clear of the brown gas. The tubes were allowed to cool and 10 ml of deionised water was added, then the digests were filtered, with washings using deionised water, through Whatman No. 50 hardened filter papers into 50 ml volumetric flasks. The solutions were then made up to volume using deionised water. The solutions were transferred to a 100 ml plastic bottles for measurement. The blank determination was carried out without soil.

2.21. TOTAL NITROGEN IN PLANT MATERIAL

The total nitrogen in plant material is determined according to the method of Bremner and Mulvaney (1982). This method does not include oxidised forms of nitrogen such as nitrate.

Samples were digested in concentrated sulphuric acid using potassium sulphate to raise the boiling point and a copper selenium catalyst mixture to accelerate the digestion process. Ammonium in the digests was determined using an automated colorimetric method on the Technicon Autoanalyzer system.

APPARATUS :

Tecator 40 tube block digester and Technicon Autoanalyzer system.

SAMPLE PREPARATION :

The vegetation samples were oven dried and finely ground using a mill.

DIGESTION PROCESS :

REAGENTS :

Analar grade reagents and nitrogen free deionised water were used throughout. These reagents were prepared in a fume cupboard. A fume cupboard was cleared before starting. Any spills were cleared up immediately and the fume cupboard was wiped down when finished.

Concentrated sulphuric acid (Analytical Reagent) :

***** Concentrated sulphuric acid is extremely corrosive. *****

A 1 litre bottle with a 5 ml dispenser was set up. The bottle was filled carefully with analytical grade concentrated sulphuric acid. Care was taken to ensure that the dispenser contained no air bubbles or the dispenser might spit when used. In addition, care was taken to ensure that the dispenser did not allow acid to siphon out of the bottle.

Kjeldahl tablets (Thompson and Capper Ltd.) :

***** Selenium is toxic by ingestion and inhalation. It is a cumulative poison. It is an animal carcinogen and there is evidence of reproductive effects. OES = 0.2 mg /m^3.*****

These tablets contained :

100 parts potassium sulphate (K_2SO_4), 6 parts copper (II) sulphate ($CuSO_4.5H_2O$) and 1 part selenium (Se).

The 5 g tablet was split in half.

***** Take care handling the catalyst tablets which contain selenium. Wear gloves. This operation must be carried out in a fume cupboard.*****

PROCEDURE :

0.2 g plant material (maximum 10 mg N) was weighed carefully to the nearest 0.1 mg approximately into a digestion tube. Care was taken to ensure that all the sample reached the bottom of the tube. This can be done using a foil weighing boat with a long detachable handle and by ensuring that the tube is carefully dried. The boat was tarred and the required amount of sample was added. This weight was recorded. Handling the weighing boat with forceps, the handle was attached to the weighing boat; and holding the digestion tube horizontally, the sample was passed to the base of the digestion tube. Using forceps, the weighing boat was removed from the handle, weighed and the weight was recorded. The difference in weight is the weight of sample transferred to the digestion tube.

Half catalyst tablet was added to each digestion tube.

***** Take care handling the catalyst tablets which contain selenium. Wear gloves. This operation must be carried out in a fume cupboard.*****

Using a dispenser, 5.0 ml (± 0.1 ml) concentrated sulphuric acid was added.

***** Take care handling the concentrated sulphuric acid as it is highly corrosive. Ensure that the dispenser contains no air bubbles or the dispenser may spit when used. Ensure that the dispenser does not allow acid to siphon out of the bottle. Point both the dispenser nozzle and the tube away from the body when dispensing the acid. Wear acid resistant gloves. This operation must be carried out in a fume cupboard.*****

The tubes were left for 15 minutes to soak the concentrated sulphuric acid so as to provide wet plant material.

***** The digestion block should be set up in a fume cupboard along with its own fume extraction system and the fume cupboard set on high.*****

The rack of tubes was placed in the digestion block and the fume extraction system was set up. The heating was gently at first until the initial frothing subsided. Plant digests are likely to froth severely in the initial stages if the initial heating is too vigorous. Once the initial carbonisation was complete, the temperature was increased to 375 °C and heating was continued until the digests clear. The baffle was placed on the front of the rack to promote refluxing higher up the tubes thereby washing material down into the tubes.

Once this was complete, the baffle was removed to prevent excessive loss and heating was continued for 1 hour.

The tubes were removed from the block and allowed to cool until just warm to the touch. If left too long, the digests may solidify and become very difficult to dissolve. Very carefully and pointing the tube towards the back of the fume cupboard, about 10 ml deionised water was added from a wash bottle.

***** The reaction on adding water to the concentrated sulphuric acid will be very vigorous and if the digests are too warm the acid may spurt from the end of the tube.*****

Once all the tubes have been diluted, a further 30 ml deionised water was added and mixed well. The contents were filtered through a Whatman No. 1 filter paper into 100 ml volumetric flasks. The tube was washed at least twice and these washings were added to the filter paper. Do not use hardened grades of filter paper as these may contain high levels of nitrogen. The volumetric flask was made up to the mark with deionised water, stoppered and mixed well. The blank determination was carried out without soil.

CHAPTER 3

METHODS DEVELOPMENT AND ASSESSMENT

3.1. AMMONIUM ANALYSIS IN SOIL AND WATER

3.1.1. INTRODUCTION

The current methods for measuring ammonium in aqueous solutions include steam distillation, microdiffusion, ion selective electrodes, fluorometric and colorimetric.

Willason and Johnson (1986) illustrated a reliable and sensitive gas diffusion technique for the rapid determination of ammonia in seawater based on the colour change in a pH indicator solution. They showed that the procedure is based on the conversion of NH_4-N in seawater to NH_3 and that following diffusion of NH_3 through a hydrophobic membrane using flow - injection analysis. The change in light transimittance of the acceptor stream produced by the ammonia due the change in the pH is measured by a light - emitting diode (LED) photometer. The magnitude of the change in the colour is proportional to the concentration of ammonia in the sample. The sampling rate of 60 samples per hour can be made on a flowing stream of seawater or discrete samples. In addition, the lower limit of detection is 0.7 μg /l. The method is ideal for mapping ammonia concentrations in estuarine, coastal and oceanic waters since it is very sensitive and automated (one sample per minute).

Aoki et al. (1986) suggested a new continuous flow method for the simultaneous determination of nitrate and ammonia in water. They reported that ammonia is fluorometrically determined with an *o*-phthalaldehyde reagent followed by the reduction of nitrate to ammonia in alkaline medium by titanium (III) chloride to determine nitrate. The separation of the ammonia generated in the alkaline sample solution is carried out with a tubular microporous PTFE membrane. Although nitrite stoichiometrically interferes but can be controlled by sulfanilic acid. Satisfactory results were obtained for the simultaneous determination of nitrate and ammonia in rivers. The limits of detection (3 standard deviations) for nitrate and ammonia were 25.2×10^{-4} and

25.2×10^{-5} mg /l, respectively. The method is so sensitive to nitrate that it gave a satisfactory result even in the case of the Uji River where the indirect photometric chromatography method failed because of insufficient sensitivity.

The colorimetric methods include the Berthelot and the Nesseler methods. These two methods have both been used in manual and automated colorimetric methods. Automated methods have the greatest accuracy and precision (Brown, 1973). In addition, since large numbers of samples can be analysed quickly and with a high degree of reproducibility, automated analysis for the determination of the inorganic nutrients in water and various soil extracting solutions is an attractive alternative to manual wet chemistry procedures (White and Gosz, 1981; and Burton et al., 1989).

The most commonly used automated technique for measuring ammonium concentration is the indophenol blue method (Berthelot method).

Searle (1984) produced a large review about the Berthelot reaction. He indicated that the so called Berthelot reaction (sometimes called the indophenol reaction) is the reaction of ammonium ions and a phenol, which results in the formation of an indophenol dye under suitable oxidising conditions in the presence of hypochlorite. The strong absorbance of the indophenol dyes lies between 630 and 720 nm since they are highly conjugated. Based on this reaction there are sensitive methods specific for the ammonium ion. These methods can also be applied to the methods in which nitrogen is converted into the ammonium form by suitable pre - treatments, such as Kjeldahl digestion. The complexity of the reaction mechanism caused problem in the early manual methods. Considerable interest in the reaction has stemmed almost entirely from the development of automated analysis, particularly continuous flow analysis (CFA) in which the reaction conditions are controllable and reproducible. Use of the automated Berthelot reaction became widespread where there are large numbers of samples for nitrogen determination. The popularity of the automated method is also because the reaction conditions are closely controlled. The most commonly used automated version of the Berthelot reaction is the nitroprusside catalysed reaction since it has high sensitivity and it reaches equilibria faster than the uncatalysed reaction. As the published automated methods are based on aqueous chemistry the phenolic compounds used are either phenol or salicylic acid because of the solubilities of their respective sodium salts. Although early workers recognised that other phenol

compounds could be used in the reaction, phenol has been the most widely used phenolic compound . These phenolic compounds include thymol, salicylic acid and its salts, α-naphthol, guaiacol, *o*-phenylphenol, *o*-chlorophenol, 2-methyl-5-hydroxyquinoline and *m*-cresol. Although phenol is cheap and readily available, it is, however, toxic by skin absorption. Phenol should be used with care since it is associated with handling hazards (poisonous, volatile) and the formation of a very unpleasantly smelling compound during the reaction. Because its solubility, low toxicity and high convenience to prepare sodium salicylate is also popular.

Although causing a rapid fading in the colour intensity with time, a development temperature of 100 °C was often used and maximum colour development occurred relatively rapidly in some of the early uncatalysed methods using phenol and hypochlorite reagents (Searle, 1984). A number of reagents have been used as catalysts for this reaction , including sodium nitrosylpentacyanoferrate (II) (sodium nitroprusside), acetone, potassium hexacyanoferrate (II) and sodium aquopentacyanoferrate (II) (Krom, 1980). Sodium nitroprusside has been established as the best catalyst for the reaction providing the greatest sensitivity (Searle, 1984).

The achievement of the formation of monochloroamine as the first stage in the reaction mechanism is obtained in the presence of hypochlorite, although the reaction proceeds through a similar mechanism when the hypochlorite is substituted, for example by organochlorine salts. The common source of hypochlorite ions is sodium hypochlorite (Searle, 1984). A number of reagents have been used as a source of hypochlorite ion, including sodium hypochlorite, sodium dichloroisocyanurate (NaDTT) and chloramine - T. Because of the stability of sodium dichloroisocyanurate a known concentration of chlorine can be provided for the reaction without the need for frequent standardisation, which is necessary if sodium hypochlorite is used (Krom, 1980). However, the rate of hydrolysis of these compounds to hypochlorite may depend on the reaction conditions. In addition, sodium hypobromite can be used. Although sodium hypochlorite is the most widely used source of hypochlorite ion, there is increasing use of organochlorine salts such as sodium dicloroisocyanurate.

Interference problems in the Berthelot reaction for nitrogen determination in ecological materials, are most likely to occur with soil samples (Rowland, 1983). When nitrogen groups such as amino, amide or amine hydrolyse to ammonia under the reaction conditions, positive interferences can arise. Interference also occurs in the

reaction in the presence of sufficient amount of some metals e.g. copper and mercury. Among the non metallic elements which cause interference are sulphur in a range of oxidation states, selenium and the halogens. The tendency of high salinity samples such as seawater to lower the reaction pH is considered to result from the precipitation of insoluble hydroxides (Searle, 1984). Furthermore, the precipitation as hydroxides at the pH of the reaction occurs due to some elements commonly found in soil extracts such as Ca, Mg, Fe and other divalent and trivalent cations. This may cause a noisy signal and results in erratic readings. Potassium sodium tartrate, trisodium citrate and EDTA have been used to eliminate the interference and prevent the precipitate from forming (Gentry and Willis, 1988).

O'Donovan (1971) found that tris (hydroxymethyl) methylamine (Tris), glutamine and phosphate inhibited the development of the indophenol colour formed by ammonia, phenol and hypochlorite, however, these compounds did not change the indophenol colour after its development.

Searcy et al. (1965) assessed the Berthelot colour reaction and showed that the sequence of adding reagents is an important parameter in the chromogenic response. The critical factor is the time lapse before the addition of the phenol reagent to the alkaline hypochlorite - containing mixtures. The volatilisation of ammonia from solutions treated with alkaline hypochlorite occurs because of the high pH. The prevention of this loss can be accomplished by adding the phenol reagent before the alkaline hypochlorite. It appears that the specificity of Berthelot colour formation can be enhanced by the phenol - hypochlorite reagent sequence.

Weatherburn (1967) examined the Berthelot colour reaction with the particular aim of presenting a simple, reliable analytical procedure. Excellent reproducibility with great convenience was obtained by using the combination of two reagents prepared readily (phenol plus nitroprusside and alkali plus hypochlorite). The hypochlorite reagent could be added up to 30 minutes after the mixing of the ammonium containing sample with the phenol reagent without change of absorbance, however, the mixing of the sample with a reagent containing hypochlorite or with alkaline phenol plus nitroprusside lead to a decrease in absorbance unless the second reagent was added immediately. The complicated interrelationship between reaction temperature, development time and nitroprusside concentration in the nitroprusside catalysed reaction makes comparisons between methods difficult, as a considerable range of nitroprusside

concentrations are used. The development of colour was satisfactory with minimal precautions as a result of using a variety of reagent concentrations and reaction temperatures of 20, 25, 37 and 75 °C. At 37 °C the development of colour was faster than at the room temperature (15 - 25 °C). Although the time to reach maximum absorbance was longer than at 37 °C, there was an increase in the sensitivity at 75 °C. The absorbance maximum was reached quickly at 100 °C, however, this temperature was not recommended because of the rapid decrease in the absorbance thereafter. As the absorbance reached a maximum rapidly and decreased abruptly, a reaction temperature of 100 °C would require critical timing and rigid duplication of standards . It is not desirable to use a temperature of 50 °C for the reaction because of the slight but continuing increase in absorbance with most concentrations of nitroprusside

Searle (1975) discussed an automated method for the measurement of ammonium ions in Kjeldahl digests and 1 M sodium chloride extracts of soils (for the determination of total nitrogen and cation exchange capacity, respectively). Based on the nitroprusside catalysed indophenol reaction, and using a Technicon Autoanalyzer II the analysis rates of 100 per hour were possible. There was a good agreement between the method and the standard distillation - titration method. Although the maximum colour development for the indophenol reaction occurred between pH 10 - 13, reaction conditions approaching pH 14 were used in order to have sufficient alkali present to neutralise the acid Kjeldahl digests. The accuracy of the Autoanalyzer method described, over the range 0 - 70 mg /l N, provided linear calibration over this range as well as it was capable of high analysis rates.

Verdouw et al. (1977) developed an ammonia determination method based on the formation of a substituted indophenol with sodium salicylate as the phenolic reagent . They reported that the salicylate method is used specifically for NH_3-N and in natural fresh water samples, interferences are generally absent, also the method can be easily applied for sea water analysis. Compared with the phenol method, salicylate is a good alternative for phenol. Although the low reactivity of the salicylate as a result of the introduction of a carboxylgroup (salicylate concentration is 4 times the phenol concentration for optimal conditions), the disadvantages of phenol, i.e. volatile reagent and o-chlorophenol formation, are much reduced, however, the sensitivity was negligibly decreased. It seems that the salicylate method has proved to be very useful since its ease in application and great reproducibility.

Krom (1980) suggested a reaction scheme (Figure 3.1.) for the spectrophotometric determination of ammonia by using a modified Berthelot reaction, in which salicylate, dichloroisocyanurate and complex cyanides are the principal reagents. He pointed out the first action of the complex cyanides in the reaction was to stabilise monochloramine at pH values (12 - 13) at which it is normally unstable and hence facilitate the formation of 5-aminosalicylate from salicylate (this step is the rate determining step of the reaction), and the second action was to accelerate the oxidative coupling of 5-aminosalicylate with salicylate to form the indophenol dye [possibly via a hexacyanoferrate (III) intermediate]. The necessity to optimise the pH value for each combination of reagents used is opened up since the optimum pH of reaction is a result of a complex inter - relationship of a number of equlibria. Since the λ_{max}. was 650 - 660 nm for all combinations of reactants the differences in the absorbencies could not have been related to measurement at a wavelength considerably different from $\lambda_{max.}$.

Rowland (1983) used the Berthelot reaction as an Autoanalyzer method for the analysis of ammonium - nitrogen. He stated that this method has a wide versatility as it can be applicable for the determination of total nitrogen in soils and plant material, and sufficiently is stable and sensitive to determine levels of NH_4-N in natural waters. The interference of amino acids contributed a relatively small fraction (11 %) in soil extracts and was not present in the soil solution examined. The use of disodium hydrogen phosphate buffer enhanced the interference from amino acids and precipitated calcium phosphate from calcareous soil extracts.

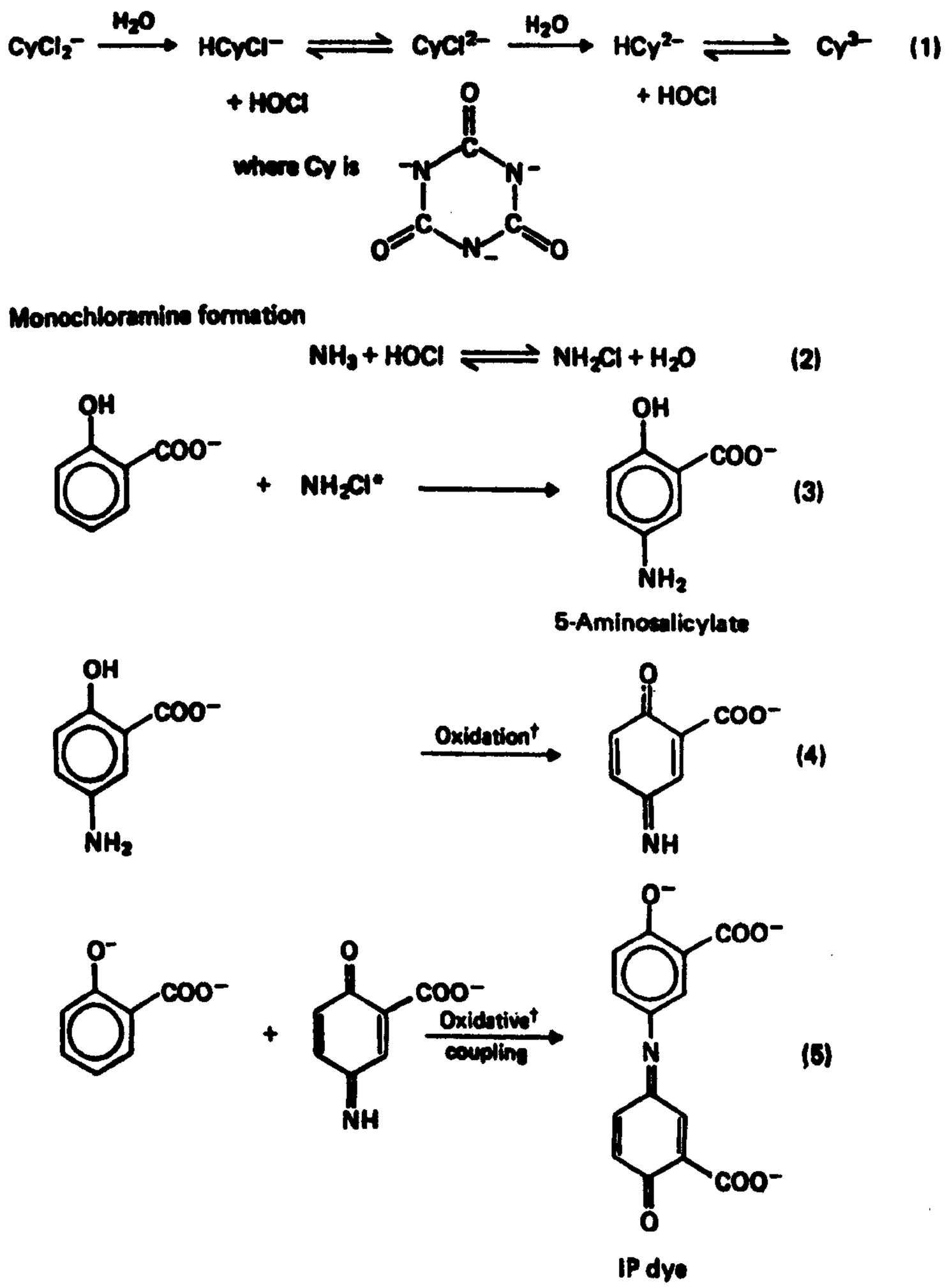


Figure 3.1. : Reaction sequence according to Krom (1980).

Markus et al. (1985) introduced the automated procedures for determining NO_2-N, NO_3-N and NH_4-N in various soil testing programs using an Autoanalyzer system equipped with a microprocessor - based controller and data handler. They reported that the new system provides improvement in accuracy and precision of results through its drift, gain, and carryover correction features. Furthermore, the new system also potentially offered a more rapid chemical determination. The determination of NO_3-N and NH_4-N in standard solutions gave a very high degree of accuracy at each of the 60, 90 and 120 per hour sampling rates studied. The variations in analytical results and the evaluated precision indicate the diminution in quality of both NO_3-N and NH_4-N

analyses at the 120 per hour sampling rate. A very close agreement in terms of the regression analysis was obtained from the comparison between the analytical results of NO_3-N and NH_4-N determined in various soil testing programs with the Autoanalyzer operated at 90 per hour sampling rate and a second, nonautomated analytical instrument. Significant advancement in the automated analysis of the above two forms of N was obtained using 90 per hour sampling rate.

Gentry and Willis (1988) tested an improved sodium salicylate - hypochlorite, automated method for ammonium in soil extracting solutions using nitroprusside as catalyst. They indicated that the sensitivity of this method is 2 to 3 times more than previously published procedures and also is less subject to interferences. In addition, the method has a no detectable base line drift , high reproducibility, there is less noise in the signal, and the method has two alternative ranges. The linearity of the method was clear for the first range with maximum sensitivity to about 2.5 mg /l, the second range to 9 mg /l. Also, they found that the efficiency of the elimination of interferences was better with sodium citrate than tartrate. Although precipitation occurred when tartrate was used at the point in the manifold where the sample mixed with ammonium reagent, it dissolved shortly thereafter. With sodium citrate no precipitate was formed, thus, further reinforcing the hypothesis that citrate is the preferred reagent for eliminating interference. No interference occurred from calcium when present at 1000 mg /l, Mg at 1000 mg /l, Fe^{+3} at 200 mg /l, or Fe^{+2} at 100 mg /l when 4 % sodium citrate was used. Despite of the slightly better results obtained when 10 % sodium citrate was used, it was felt that the improvement was not sufficient to justify using additional quantities of sodium citrate.

Doyle and Schimel (1996) concluded that the salicylate indophenol reaction has a pH optimum at salicylate's pKa (13.4) under conditions of high ionic strength and elevated temperature in continuous flow analysis. The developed method is applicable for acidic solutions up to 5 N at 60 samples per hour due to its rapid colour development, high buffering capacity, moderate flow rate, and low detection limit. Furthermore, this method is more reliable than most commonly - used salicylate chemistries for analysing ammonium in acid digests, especially at low levels needed for microbial biomass extracts. Raising the temperature from 30 °C to 60 °C doubled the indophenol absorbance. Although the acid interference was accentuated by using higher temperature, it was reduced by using a long (13 m) coil. Thermal stability was better

with using water bath than using the originally provided aluminium block heater. Residence time in the water bath was approximately 40 s. Despite the less stability of the indophenol colour at high temperature (Searle, 1984), the colour development was accelerated at high temperature which is useful with rapid automated analysis.

White and Gosz (1981) compared an automated high temperature uncatalysed (Technicon industrial method No. 98 - 70w) phenol - hypochlorite method for determination of inorganic NH_4-N with the steam distillation method for analysis of KCl extracts of forest floor samples. They showed that the addition of amino acids to KCl extracts contributed significant positive interference in the automated method. When the automated method was modified by using 110 g NaOH /l instead of 200 g NaOH /l in the phenol reagent (which lowered the pH to 12.5 in the reagent and to 12.0 ± 0.1 in the final solution) and by lowering the temperature from 90 to 60 °C in the heating bath, similar results to that of steam distillation analysis were obtained. The original method overestimated inorganic NH_4-N in extracts of forest floor material by 17 - 26 %.

Burton et al. (1989) investigated the potential interference with ammonium determination from co-extracted amino acids using the uncatalysed phenol - hypochlorite, high temperature method. Pure solutions of 22 amino acids were subjected to ammonium determination by both the indophenol method and steam distillation to test the extent of colour development. Although the indophenol procedure gave apparent detection of amino acid as ammonium ranged from 0 to 94 % of total nitrogen, the steam distillation resulted in little apparent ammonium recovery. With the exception of therionine, the extent of colour development was inversely attributed to amino acid molecular weight. Both the size and composition of the co-extracted amino acid pool is important in determining the extent of interference due to the range in recoveries for the indophenol procedure . The increased interference was explained by the release of amino acid as a result of pre-treatment. Comparison showed that the difference between distillation and indophenol estimates of ammonium content of 0.5 M K_2SO_4 was dependent upon ammonium content. It is recommended to use the procedures employing a distillation step (manual or automated) to avoid amino acid interference when precise NH_4-N determinations are needed on dried or fumigated samples.

Searle (1990) indicated that the Berthelot reaction can be used for measuring ammonium ions in the presence of appreciable concentrations of amino acids, provided

that care should be taken in choosing the reagents and reaction conditions used to limit hydrolysis. The use of nitroprusside catalysed reaction, which enables the use of low samples volumes, reagent concentrations and reaction temperatures gave the best achievement. Comparison demonstrated that the nitroprusside catalysed reaction used originally for the analysis of Kjeldahl total nitrogen extracts, causes significantly less hydrolysis of amino acids than the Technicon method as used by Burton et al. (1989). As the method was modified by lowering the NaOH concentration and reaction temperature , hydrolysis was reduced to the point where it is insignificant given that the concentrations of amino acids used in this study are unlikely to occur in 2 M KCl soil extracts. Significant decreases in reaction sensitivity and also other problems may occur as the optimisation of the reaction conditions to further limit hydrolysis is probably feasible. For instance, negative interferences through reactions between amino acids and the hypochlorite source may occur in the presence of very low hypochlorite concentrations (Searle, 1984). However, no negative interference occurred when the concentration of NaDTT was used in this modified method.

In this department, ammonium is measured on a Technicon Autoanalyzer II system using a variation of the Berthelot method based on Brown (1973). This method is a phenol - hypochlorite method using sodium nitroprusside as a catalyst and potassium sodium tartrate and sodium citrate to eliminate the interferences caused by the divalent and trivalent cations. Khan (1994) examined the interferences caused by the amino acids in the determination of ammonium - N by the Berthelot colour reaction and found that the organic compounds such as amino acids caused both positive and negative interferences in the determination of ammonium - N. As the reagent concentrations and the reaction conditions have not been optimised, it was, decided to conduct experiments to optimise this method. The purpose of this study was to :

1- To optimise the reagent concentrations to maximise the sensitivity of the method.

2- To optimise the pH of the reaction.

3- To optimise the temperature of the manifold water bath.

4- To determine the absorbance spectrum of the indophenol product.

5- To evaluate the optimised method for the determination of ammonium in soil extracts and water samples in terms of the precision and sensitivity.

3.1.2. MATERIALS AND METHODS

3.1.2.1. METHOD DEVELOPMENT

3.1.2.1.1. SAMPLE FLOW RATE

In order to increase the sensitivity of the method, the sample flow rate was changed from 0.23 to 0.32 ml /min. and the hypochlorite flow rate was reduced from 0.23 to 0.16 ml/min. The hypochlorite concentration was increased in proportion. The other reagent concentrations and flow rates were not changed. The flow rates of the new system are presented in Table 3.1.

Table 3.1. : Reagent flow rate of the new system used in the optimisation of the standard method for the ammonium-N determination.

Tube	Flow rate (ml /min.)
Sample	0.32
Hypochlorite	0.16
Complexing Reagent	0.60
Alkaline Phenol	0.60
Air	0.32
Pull Through	1.20

3.1.2.1.2. OPTIMISATION OF THE REAGENTS, REACTION CONDITIONS AND THE SELECTION OF THE SUITABLE METHOD OF ANALYSIS

Analar grade reagents and nitrogen free deionised water were used in the preparation of all solutions. The hypochlorite and phenol are usable for up to 14 days after preparation, providing they are stored in dark reagent bottles. Nitroprusside ion slowly decomposes. Hence it is preferable to prepare fresh regents at least once fortnightly. An advantage of these reagents is that they are two to four times more dilute than those previously used. An important factor when such corrosive materials are used (Brown, 1973). The reagents are stored at the room temperature except that the standard solutions were stored at 2 °C.

This optimisation was carried out by two different methods of analysis :

(a)- INDIVIDUAL CONCENTRATION METHOD :

In this method the reagents and the reaction conditions were kept constant , one reagent was chosen to be tested and different concentrations were prepared . These different concentrations were run individually with 1.0 mg /l NH_4-N standard solution to get the optimum concentration of this reagent.

PROCEDURE :

The start - up procedure was followed and the system was allowed to equilibrate with a particular test solution concentration. When the chart tracing reached a stable base line, 1.0 mg /l NH_4-N standard solution was run with STD CAL set at 1. This was repeated for each test concentration.

(b)- GRADIENT CONCENTRATION METHOD :

In this method there is a 250 ml reservoir beaker containing a constant volume of deionised water (100 ml). There is Flow In containing the concentrated test reagent and there is Flow Out going to the system containing the reagent whose concentration increases gradually with time. The flow rate was the same to maintain an equal volume in the reservoir beaker. This is shown in Figure 3.2.

The concentration of the reagent at any time can be calculated from the following equation :

$$R = C \left[1 - e^{\frac{-Ft}{V}} \right]$$

Where :

R = Reagent concentration

F = Flow rate

V = 100 ml

t = Time (the time required for drawing 0.5 inch of the peak = 6.0 minutes and 30 seconds).

C = Concentration of the concentrated test reagent.

PROCEDURE :

The start - up procedure was followed and the system was allowed to equilibrate. When the chart tracing reached a stable base line, 1.0 mg /l NH_4-N standard solution was run with STD CAL set at 1 with the increasing concentration of the test reagent.

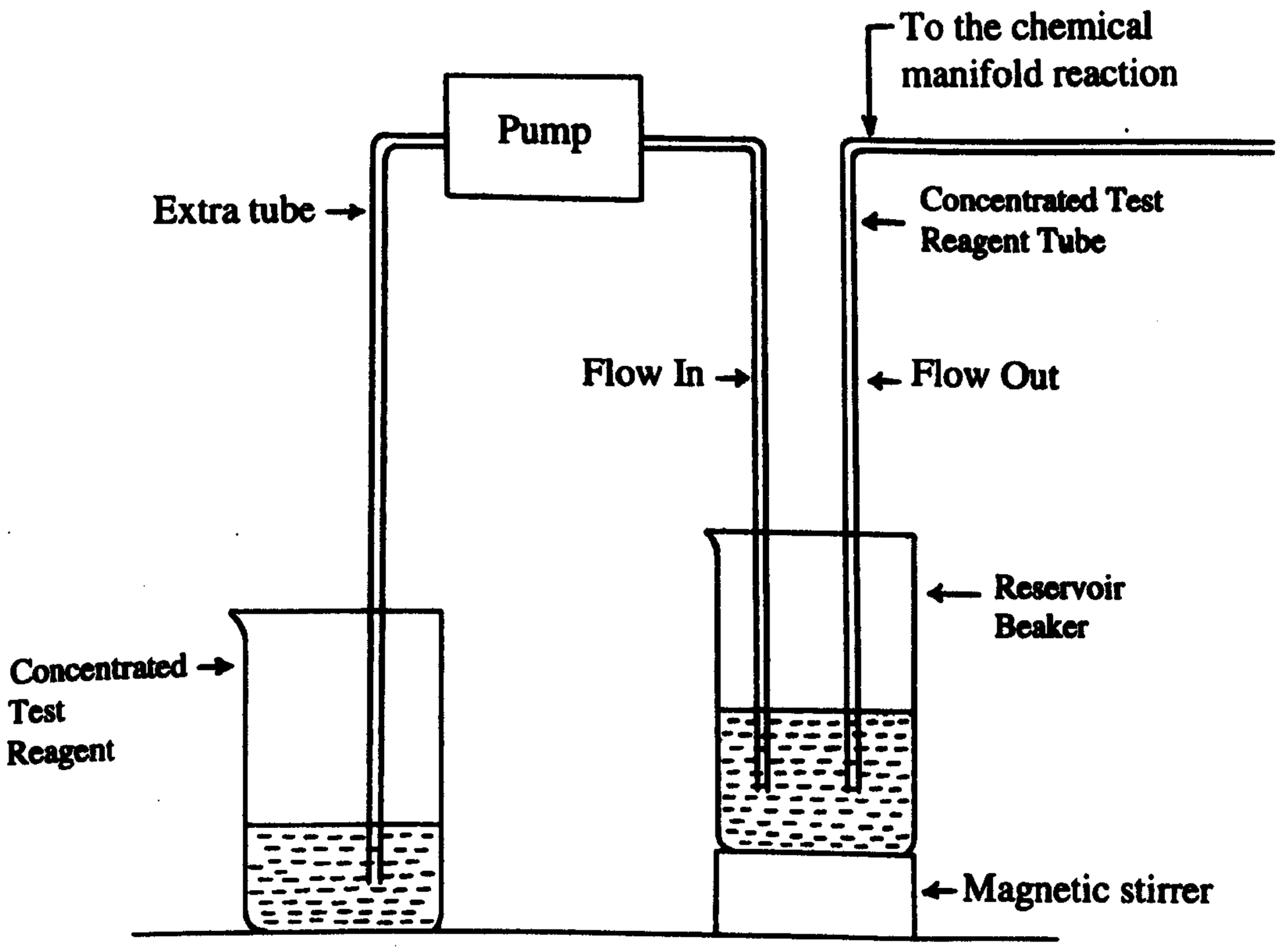


Figure 3.2. : System for continuous gradient method.

3.1.2.1.2.1. OPTIMISATION OF THE PHENOL CONCENTRATION

The following individual concentrations of phenol were tested :

0.0, 30, 40, 50, 60 g phenol /litre of sodium hydroxide solution. The NaOH solution was standardised at 22.5 g NaOH / litre deionised water. All the reaction

conditions and other non optimised reagents were kept constant throughout. This test was carried out in one run.

3.1.2.1.2.2. OPTIMISATION OF THE SODIUM HYDROXIDE CONCENTRATION AND THE pH

The following individual concentrations of sodium hydroxide were tested : 15, 20, 22.5, 25, 30, 35 g sodium hydroxide / litre of alkaline phenol solution. The phenol solution was standardised at 50 g /l. All other reaction conditions and other non optimised reagents were kept constant throughout. This test was carried out in one run.

The pH was measured for each solution of these sodium hydroxide tested solutions by the pH meter.

PROCEDURE :

The pH meter was connected to the system as shown in Figure 3.3. The pH meter and a combined glass / reference electrode was first standardised with buffer solutions of pH 7 and 4. The start - up procedure was followed and the system was allowed to equilibrate. When the chart tracing reached a stable base line, 1.0 mg /l NH_4-N standard solution was run with STD CAL set at 1. pH was monitored continuously throughout each run for the different NaOH solutions.

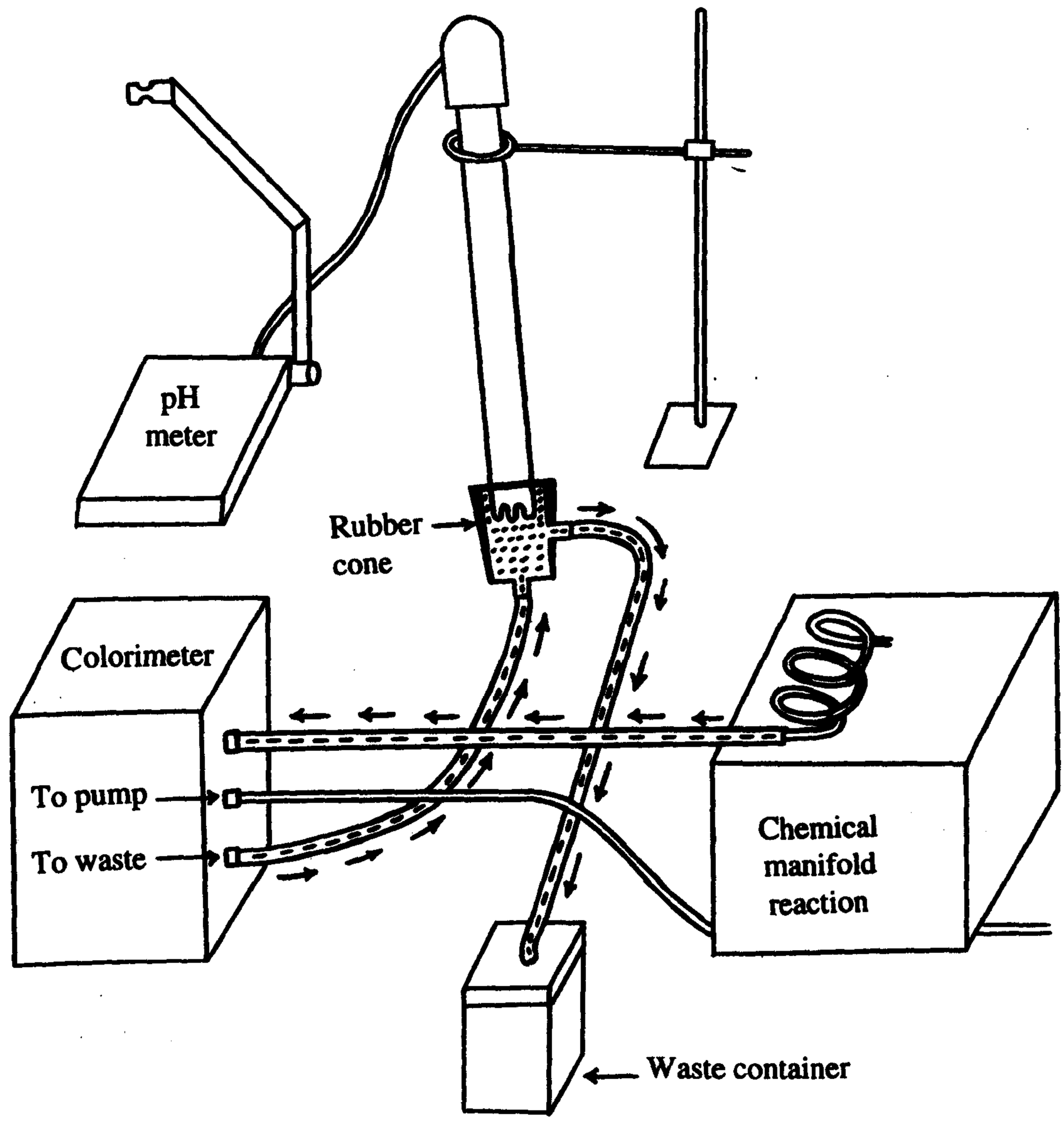


Figure 3.3. : Flow cell for measuring pH of the flow stream at the colorimeter outlet.

3.1.2.1.2.3. <u>OPTIMISATION OF POTASSIUM SODIUM TARTRATE AND SODIUM CITRATE CONCENTRATIONS</u>

The following individual concentrations of potassium sodium tartrate and sodium citrate were tested :

30 g potassium sodium tartrate + 30 g sodium citrate, 40 g potassium sodium tartrate + 40 g sodium citrate, 50 g potassium sodium tartrate + 50 g sodium citrate, 60 g potassium sodium tartrate + 60 g sodium citrate and 70 g potassium sodium tartrate + 70

g sodium citrate in litre of sodium nitroprusside solution. The sodium nitroprusside solution was standardised at 0.5 g sodium nitroprusside and the wetting agent was added at 1 ml 15% Brij.-35 solution /litre. All the reaction conditions and other non optimised reagents were kept constant throughout. This test was carried out in two runs.

3.1.2.1.2.4. OPTIMISATION OF THE SODIUM NITROPRUSSIDE CONCENTRATION

INDIVIDUAL CONCENTRATION METHOD :

The following individual concentrations of sodium nitroprusside were tested. These concentrations of sodium nitroprusside were normalised on common base and were listed as follows :

0.2, 0.25, 0.3, 0.4, 0.5, 0.6, 1.0, 1.5 and 2.0 g sodium nitroprusside / litre of complexing reagent solution. The complexing reagent solution was standardised at 50 g potassium sodium tartrate + 50 g sodium citrate and the wetting agent was added at 1 ml 15% Brij.-35 solution /litre. All the reaction conditions and other non optimised reagents were kept constant throughout. This test was carried out in 3 runs.

A further test was carried out to study the effect of different concentrations of sodium nitroprusside on the base line by using 0.0 mg /l NH_4-N standard solution . The following individual concentrations of sodium nitroprusside were tested : 0.2, 0.3, 0.4, 0.5 and 0.6 g sodium nitroprusside / litre of the complexing reagent solution. All the reaction conditions and other non optimised reagents were kept constant. This test was accomplished in two runs. Each run was done as above.

GRADIENT CONCENTRATION METHOD :

An increasing concentration of sodium nitroprusside was tested. This increasing concentration of sodium nitroprusside was obtained using the following :

Flow In, Flow Out (0.6 ml/min.).

Reservoir Beaker contained 100 ml of complexing reagent solution (free of sodium nitroprusside) which was standardised at 5 g potassium sodium tartrate + 5 g sodium citrate and the wetting agent was added at 0.1 ml 15% Brij.-35 solution / 100 ml deionised water.

Concentrated test reagent was 100 ml of complexing reagent solution (concentrated sodium nitroprusside) which was standardised at 5 g potassium sodium tartrate + 5 g sodium citrate + 1 g sodium nitroprusside and the wetting agent was added at 0.1 ml 15% Brij.-35 solution / 100 ml deionised water.

All the reaction conditions and other non optimised reagents were kept constant throughout. This test was carried out in 2 runs.

A further test was carried out to study the effect of the increasing concentration of sodium nitroprusside on the base line by using 0.0 mg /l NH_4-N standard solution. This test was carried out in 2 runs.

3.1.2.1.2.5. OPTIMISATION OF THE SODIUM HYPOCHLORITE CONCENTRATION

INDIVIDUAL CONCENTRATION METHOD :

The following individual concentrations of sodium hypochlorite were tested :

5, 10, 20, 30, 40, 50, 60 and 70 ml sodium hypochlorite solution / litre. All the reaction conditions and other non optimised reagents were kept constant throughout. This test was carried out in 3 runs.

GRADIENT CONCENTRATION METHOD :

An increasing concentration of sodium hypochlorite was tested. This increasing concentration of sodium hypochlorite was obtained using the following :

Flow In, Flow Out (0.16 ml /min.).

Reservoir Beaker contained 100 ml deionised water.

Concentrated test reagent was 50 ml of concentrated sodium hypochlorite solution.

All the reaction conditions and other non optimised reagents were kept constant throughout. This test was carried out in 2 runs.

A further test was done to study the effect of the increasing concentration of sodium hypochlorite on the base line by using 0.0 mg /l NH_4-N standard solution. This test was done in one run.

3.1.2.1.2.6. ABSORPTION SPECTRUM OF THE COLOURED PRODUCT

PROCEDURE :

While measuring 1.0 mg /l NH_4-N standard solution, a 10 ml sample of the indophenol product was collected from the colorimeter outlet. The absorbance spectrum was measured using the spectrophotometer (SHIMADZU - Model : UV 3101PC). All other reaction conditions and the optimised reagents were kept constant throughout.

3.1.2.1.2.7. OPTIMISATION OF THE TEMPERATURE OF THE MANIFOLD WATER BATH

The following temperatures of the manifold water bath were tested :

10, 15, 21, 25.5, 30, 35.5, 40.5 and 45 C.

All other reaction conditions and the non optimised reagents were kept constant throughout. This test was carried out in one run.

PROCEDURE :

The start - up procedure was followed and the system was allowed to equilibrate with a particular temperature of the manifold water bath. When the chart tracing reached a stable base line, 1.0 mg /l NH_4-N standard solution was run with STD CAL set at 1. This was repeated for each manifold water bath temperature.

3.1.2.1.3. ASSESSMENT OF THE PRECISION OF THE OPTIMISED METHOD FOR THE AMMONIUM DETERMINATION

20 samples of 1.0 and 5.0 mg /l NH_4-N standard solutions were analysed separately using the Autoanalyzer sampler at 40 samples per hour.

The statistical analysis for the results was carried out by the Minitab Program (Release 9.2).

3.1.3. RESULTS AND DISCUSSION

3.1.3.1. OPTIMISATION OF REAGENTS, REACTION CONDITIONS AND THE SELECTION OF THE SUITABLE METHOD OF ANALYSIS

3.1.3.1.1. OPTIMISATION OF THE PHENOL CONCENTRATION

The effect of different concentrations of phenol on the sensitivity of the standard ammonium method is presented in Figure 3.4. As can be seen from the figure, the phenol concentration has an effect on the sensitivity. After an initial increase, the sensitivity of the method is decreased by the high concentration of phenol (60 g /l which is equal to 21.43 g /l in the final solution of the system).

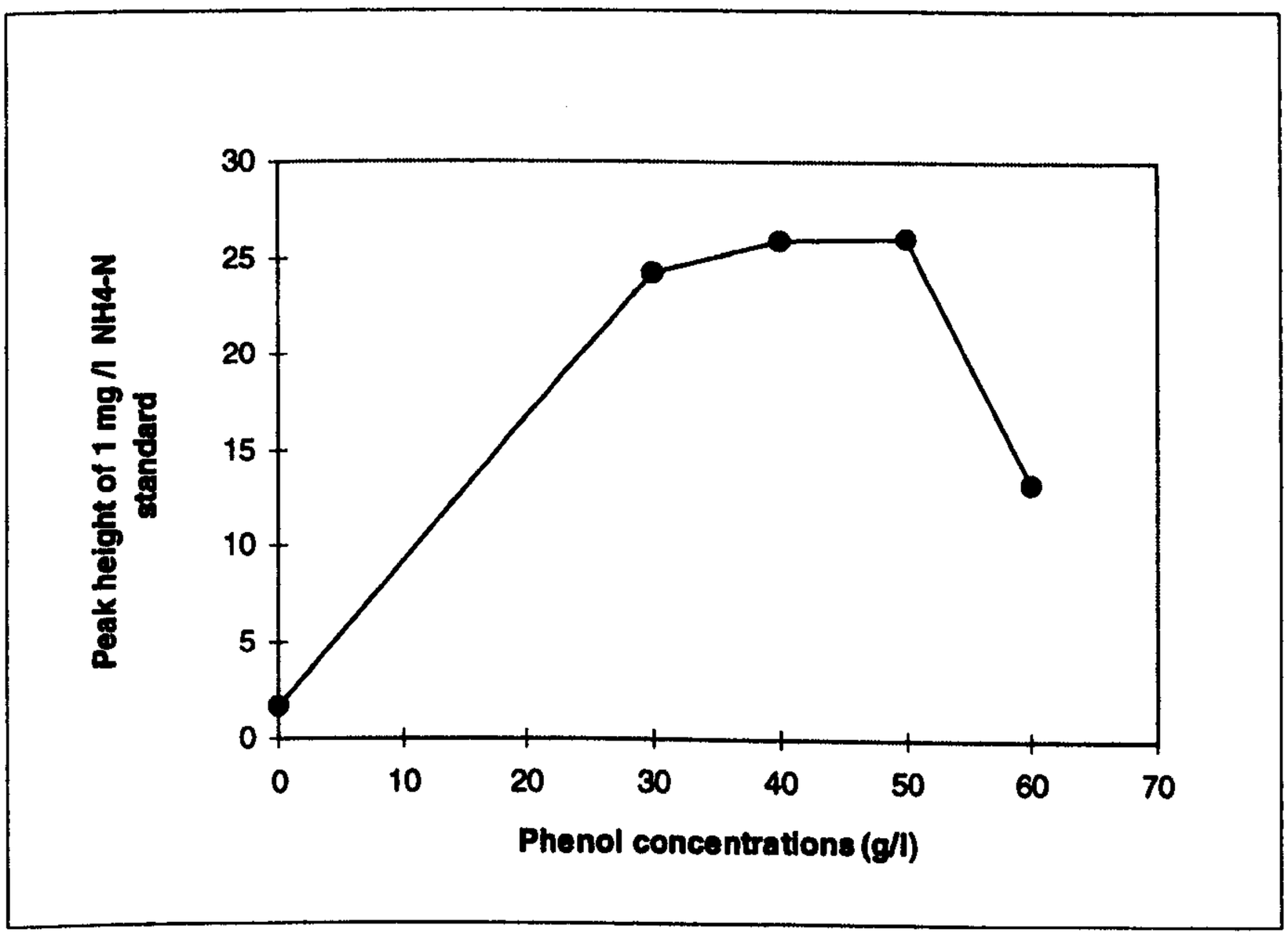


Figure 3.4. : Effect of different concentrations of phenol on the sensitivity of the standard ammonium-N method.

It is recommended that the optimum concentration of phenol is 45 g /l, which is equal to 16.07 g /l in the final solution of the system. A similar result was obtained by Harwood and Huyser (1970) who recommended the optimum concentration of phenol to be 17 g /l in the final solution.

Other authors suggested different concentrations of phenol as shown in Table 3.2.

Table 3.2. : Phenol concentrations of different authors for the sodium nitroprusside catalysed phenol-hypochlorite method.

Author	Phenol concentration* (g /l)
Brown (1973)	4.95
Berg and Abdullah (1977)	4.62
Mantoura and Woodward (1983)	3.97
Tel and Jansen (1992)	12.02
Technicon Autoanalyzer (Industrial method #624-81W GT)	16.23

* Phenol concentration is in g phenol /l in the final solution.

3.1.3.1.2. OPTIMISATION OF SODIUM HYDROXIDE CONCENTRATION

Figure 3.5. shows the effect of different concentrations of sodium hydroxide on the sensitivity of the standard ammonium method. It was observed from the figure that the optimum concentration of NaOH is 22.5 g /l which is equal to 8.03 g /l in the final solution of the system but the selected concentration of NaOH is 25.0 g /l which is equal to 8.93 g /l in the final solution because the curve was less steep at this point and the slope decreased abruptly below 22.5 g /l.

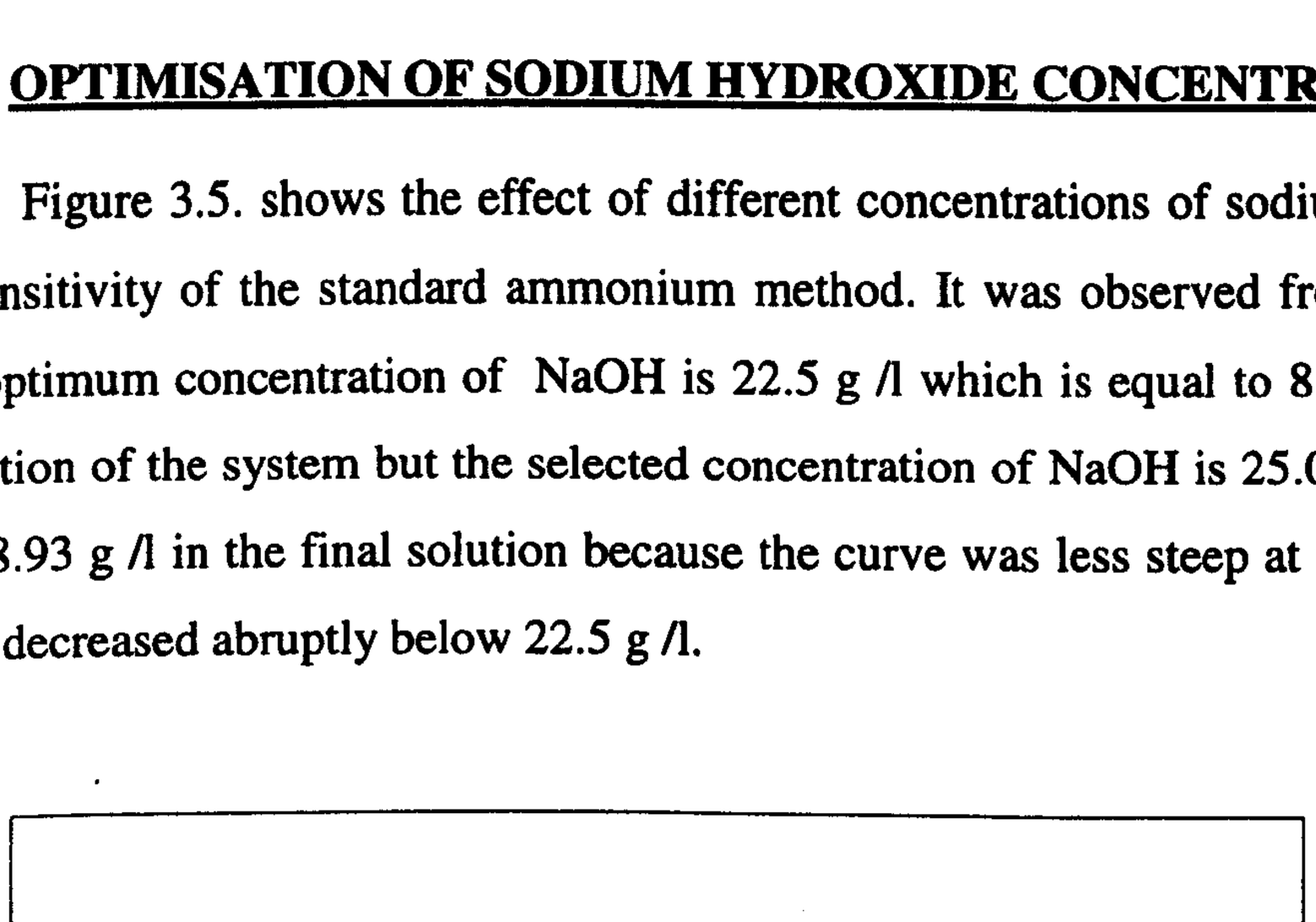


Figure 3.5. : Effect of different concentrations of sodium hydroxide on the sensitivity of the standard ammonium-N method.

Other authors suggested different concentrations of sodium hydroxide as shown in Table 3.3. The selected concentration is higher than in any of these other methods.

Table 3.3. : Sodium hydroxide concentrations of different authors for the sodium nitroprusside catalysed phenol-hypochlorite method.

Author	Sodium hydroxide concentration* (g /l)
Brown (1973)	2.60
Berg and Abdullah (1977)	1.17
Mantoura and Woodward (1983)	1.91
Tel and Jansen (1992)	6.95
Technicon Autoanalyzer (Industrial method #624-81W GT)	6.36

* Sodium hydroxide concentration is in g sodium hydroxide /l in the final solution.

3.1.3.1.3. OPTIMISATION OF THE pH

Searle (1984) pointed out that as the reagents , catalysts and concentrations used in the Berthelot reaction affects on the optimum pH reaction, many optimum pH values have been suggested.

The influence of the pH on the sensitivity of the standard ammonium method is presented in Figure 3.6. It can be seen from the figure that the optimum pH is 11.7 but the pH value 12.1 is corresponded to the selected concentration of NaOH (25 g /l, which is equal to 8.93 g /l in the final solution). Similar result was obtained by Harwood and Huyser (1970) who indicated that pH value 12.3 gave the maximum of the indophenol-blue colour development but pH value between 11.4 and 12.3 resulted in an essentially stable colour. But lower pH optima were suggested by Mantoura and Woodward (1983); pH of approximately 10.6 and Krom (1980); pH 10.7 - 11.1.

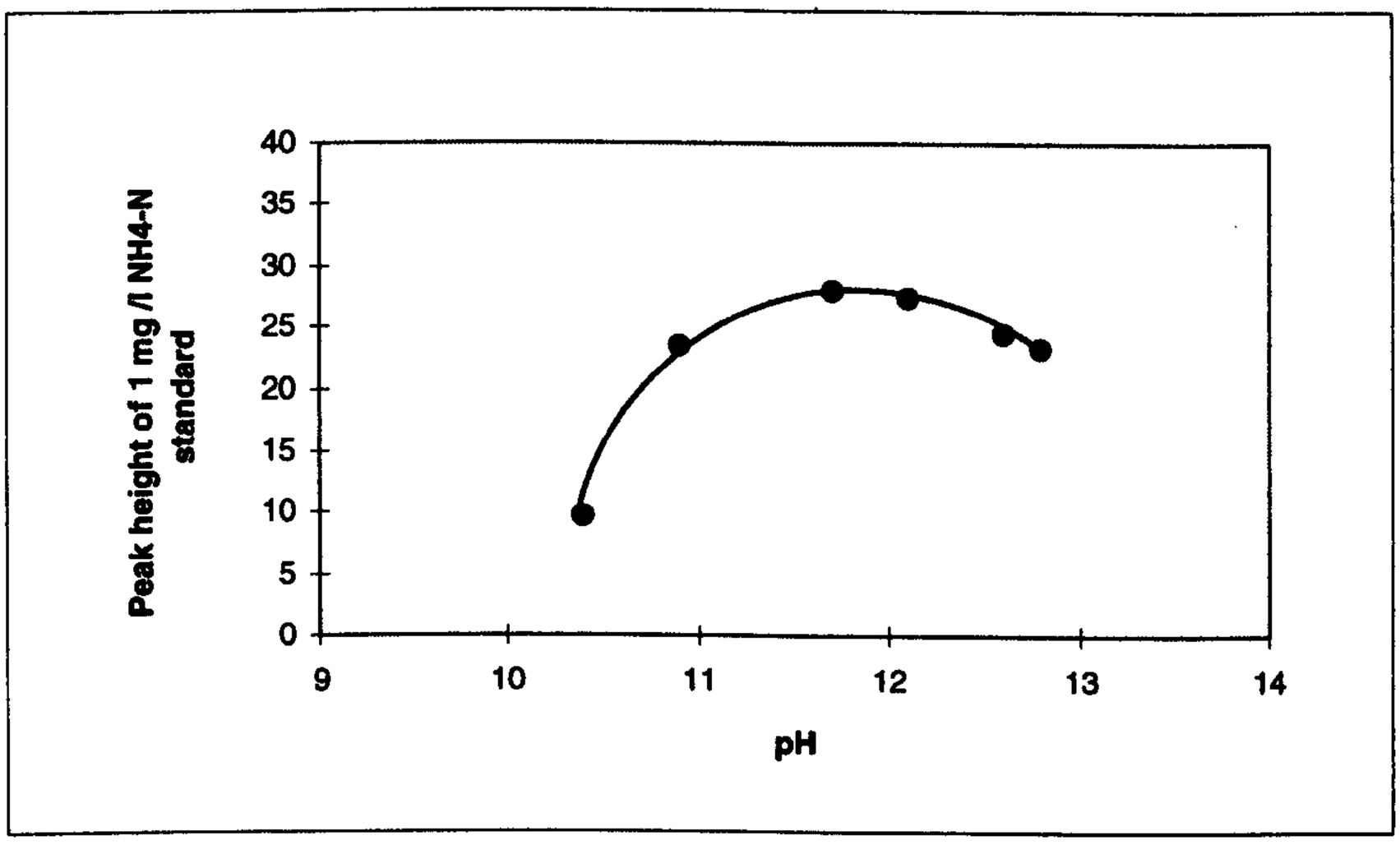


Figure 3.6. : Effect of the pH on the sensitivity of the standard ammonium-N method.

A higher pH values optima are often suggested when salicylic acid is used [Krom (1980); pH 12 -13 and Doyle and Schimel (1996); pH 13.4].

3.1.3.1.4. <u>OPTIMISATION OF THE POTASSIUM SODIUM TARTRATE AND SODIUM CITRATE CONCENTRATIONS</u>

The effect of different concentrations of potassium sodium tartrate and sodium citrate on the sensitivity of the standard ammonium method is shown in Figure 3.7. It can be noticed from the figure that there is a very slight decrease in the slope of the peak, therefore, the potassium sodium tartrate and sodium citrate concentrations have no effect on the sensitivity.

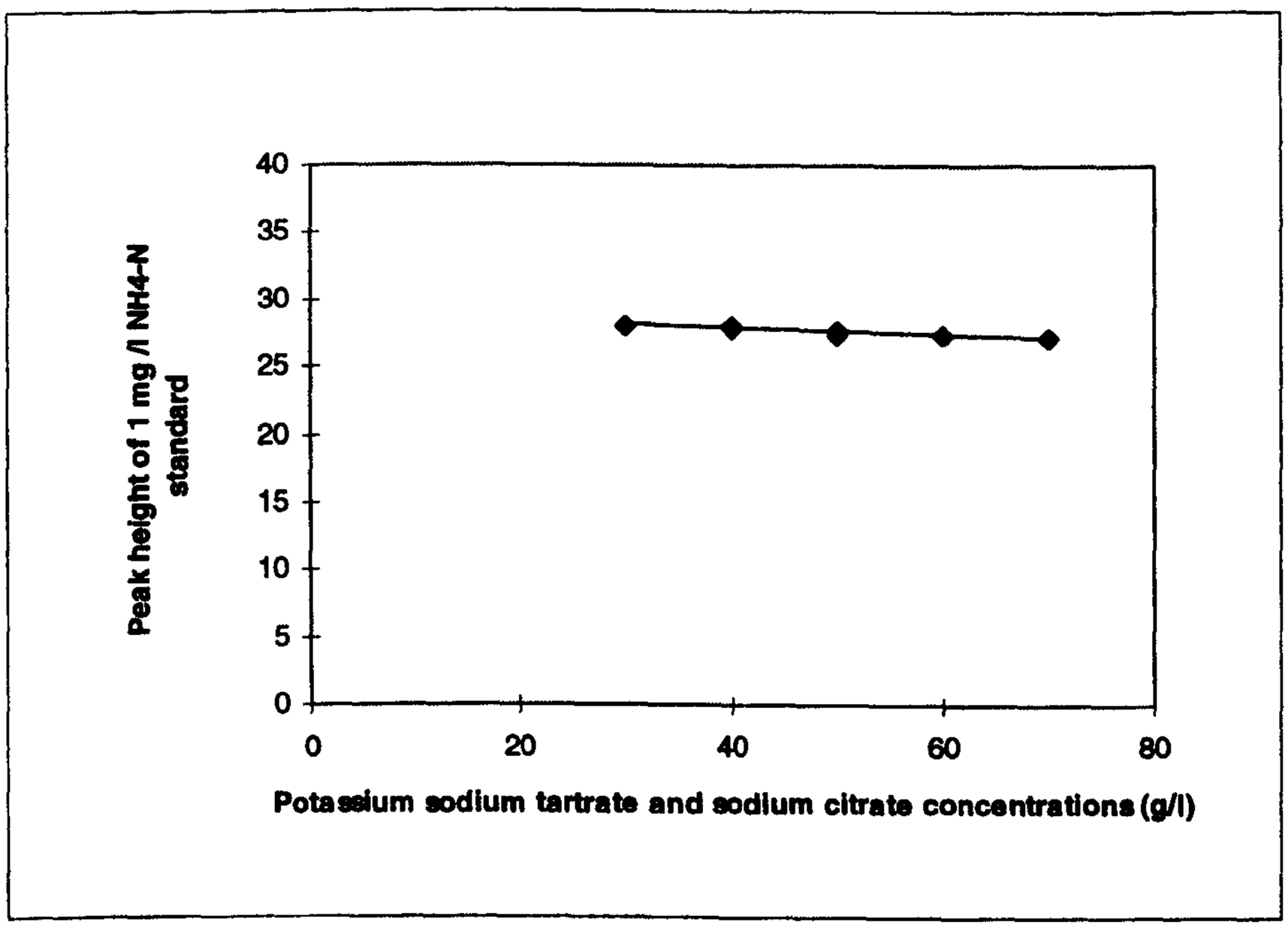


Figure 3.7. : Effect of different concentrations of potassium sodium tartrate and sodium citrate on the sensitivity of the standard ammonium-N method.

A concentration of potassium sodium tartrate and sodium citrate is 50 g /l, which is equal to 17.86 g /l for each in the final solution of the system, was selected.

Other authors suggested different concentrations of potassium sodium tartrate and sodium citrate as shown in Table 3.4.

Table 3.4. : Potassium sodium tartrate and sodium citrate concentrations of different authors for the sodium nitroprusside catalysed phenol-hypochlorite method.

Author	**Potassium sodium tartrate concentration* (g /l)**	**Sodium citrate concentration* (g /l)**
Berg and Abdullah (1977)	--------	23.49
Mantoura and Woodward (1983)	--------	16.67
Tel and Jansen (1992)	--------	--------
Technicon Autoanalyzer (Industrial method #624-81W GT)	11.09	8.07

* Potassium sodium tartrate and sodium citrate concentrations are in g /l in the final solution.

Gentry and Willis (1988) used two different concentrations of 6.6 g /l and 16.5 g /l in the final solution for both the potassium sodium tartrate and sodium citrate in the salicylate method. They found that the efficiency of the elimination of interferences was much better with sodium citrate than with potassium sodium tartrate. With potassium sodium tartrate, precipitation occurred at the point in the manifold where the sample was mixed with ammonium reagent but dissolved shortly thereafter. With sodium citrate in place of potassium sodium tartrate, no precipitate was formed, thus, further reinforcing the hypothesis that citrate is the preferred reagent for eliminating interference. No interference occurred from Ca when present at 1000 mg /l, Mg at 1000 mg /l, Fe^{+3} at 200 mg /l, or Fe^{+2} at 100 mg /l with the use of 6.6 g /l sodium citrate. Despite of the slightly better results obtained when 16.5 g /l sodium citrate was used, it was felt that the additional quantities of sodium citrate were not justified since they resulted in insufficient improvement.

3.1.3.1.5. OPTIMISATION OF SODIUM NITROPRUSSIDE CONCENTRATION

(a)- INDIVIDUAL CONCENTRATION METHOD :

Figure 3.8. shows the effect of different concentrations of sodium nitroprusside on the sensitivity of the standard ammonium method. It is obvious from the figure that the sodium nitroprusside concentration has no effect on the sensitivity at the levels tested. The very slight decrease in the slope may be due to the fact that there is interaction between the required time for developing the colour and the concentration of sodium nitroprusside. It is supposed that the optimum concentration of sodium nitroprusside used will increase as the time period for developing the colour decreases.

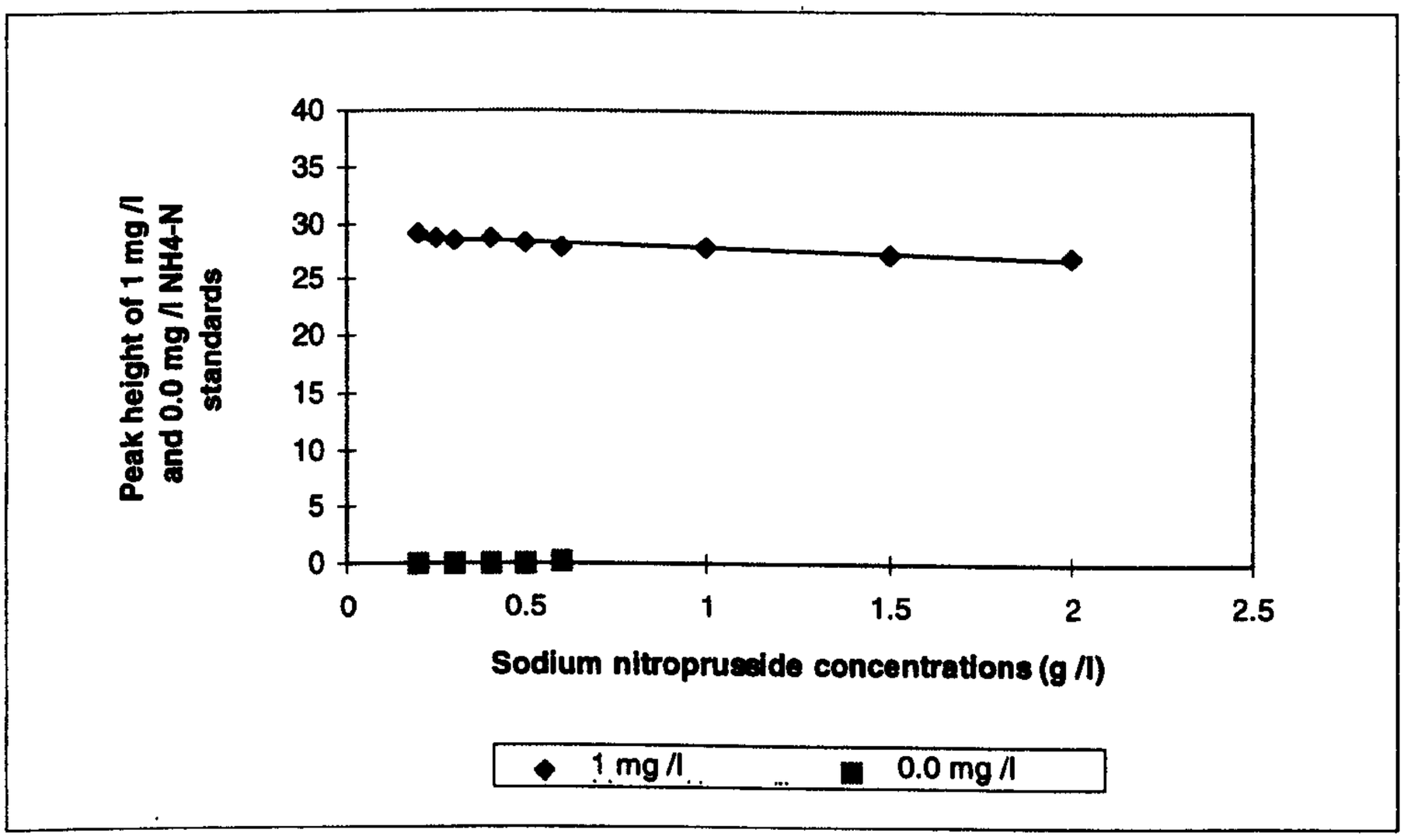


Figure 3.8. : Effect of different concentrations of sodium nitroprusside on the sensitivity of the standard ammonium-N method.

As there is a very slight change in the slope, the optimum concentration of sodium nitroprusside can be anywhere in the slope but the chosen concentration is 0.5 g /l, which is equal to 0.18 g /l in the final solution of the system, because the base line slope has the same behaviour as that of 1.0 mg /l NH_4-N standard solution. In addition, to choose high concentration of sodium nitroprusside as optimum concentration will cost much.

(b)- <u>GRADIENT CONCENTRATION METHOD :</u>

Figure 3.9. shows a graph drawn between the peak height of a 1.0 mg /l NH_4-N standard solution and run time (minutes). Figure 3.10. shows a graph drawn between 1.0 mg /l and 0.0 mg /l NH_4-N standard solutions and the concentration (g /l) of sodium nitroprusside. The concentration of sodium nitroprusside was calculated using the equation described in section 3.1.2.1.2. The base line increases with increasing sodium nitroprusside concentration, therefore, the values for 1.0 mg /l NH_4-N have been corrected by the subtraction of the baseline value.

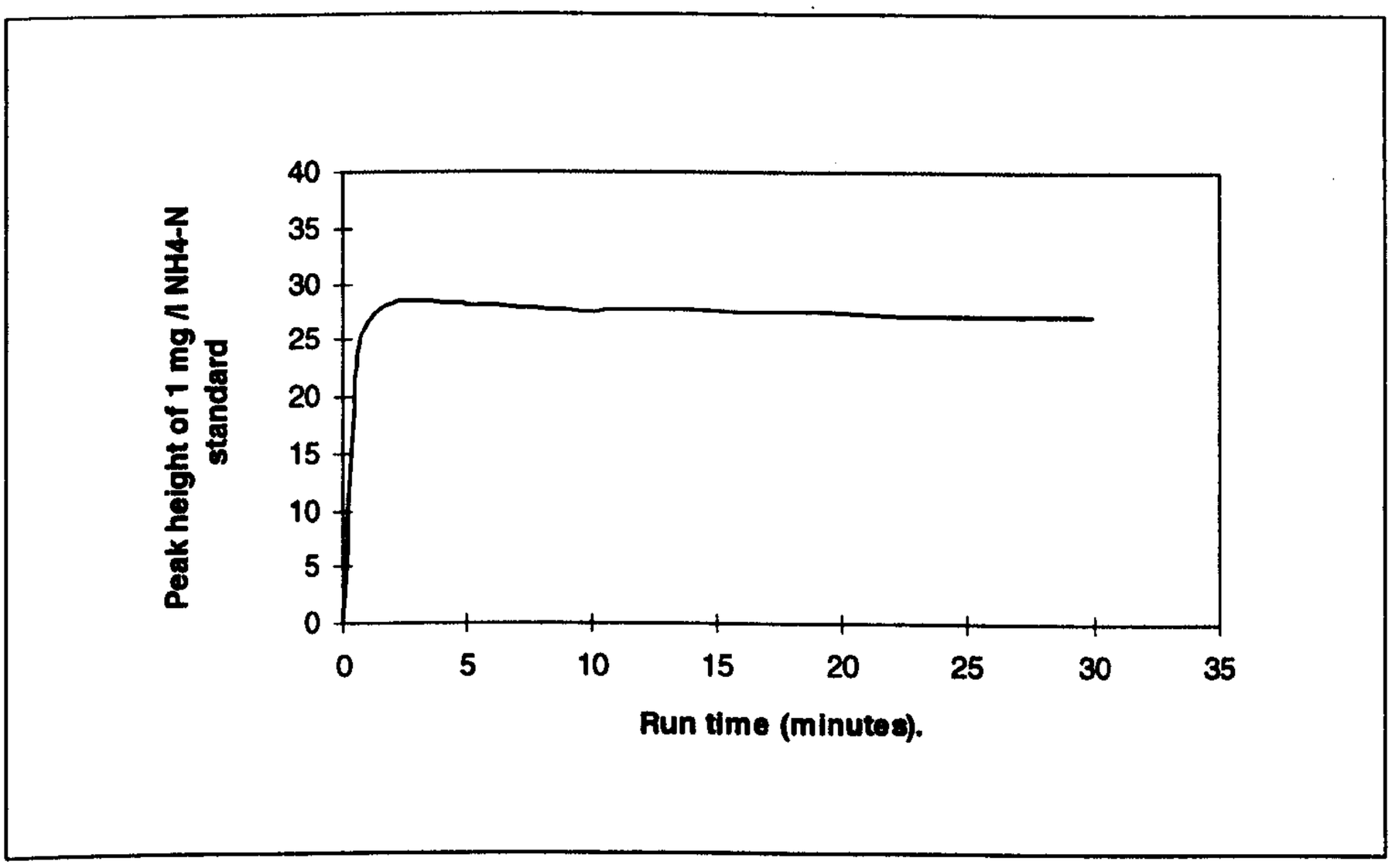


Figure 3.9. : Effect of sodium nitroprusside concentration on the sensitivity of the standard ammonium-N method using the gradient method.

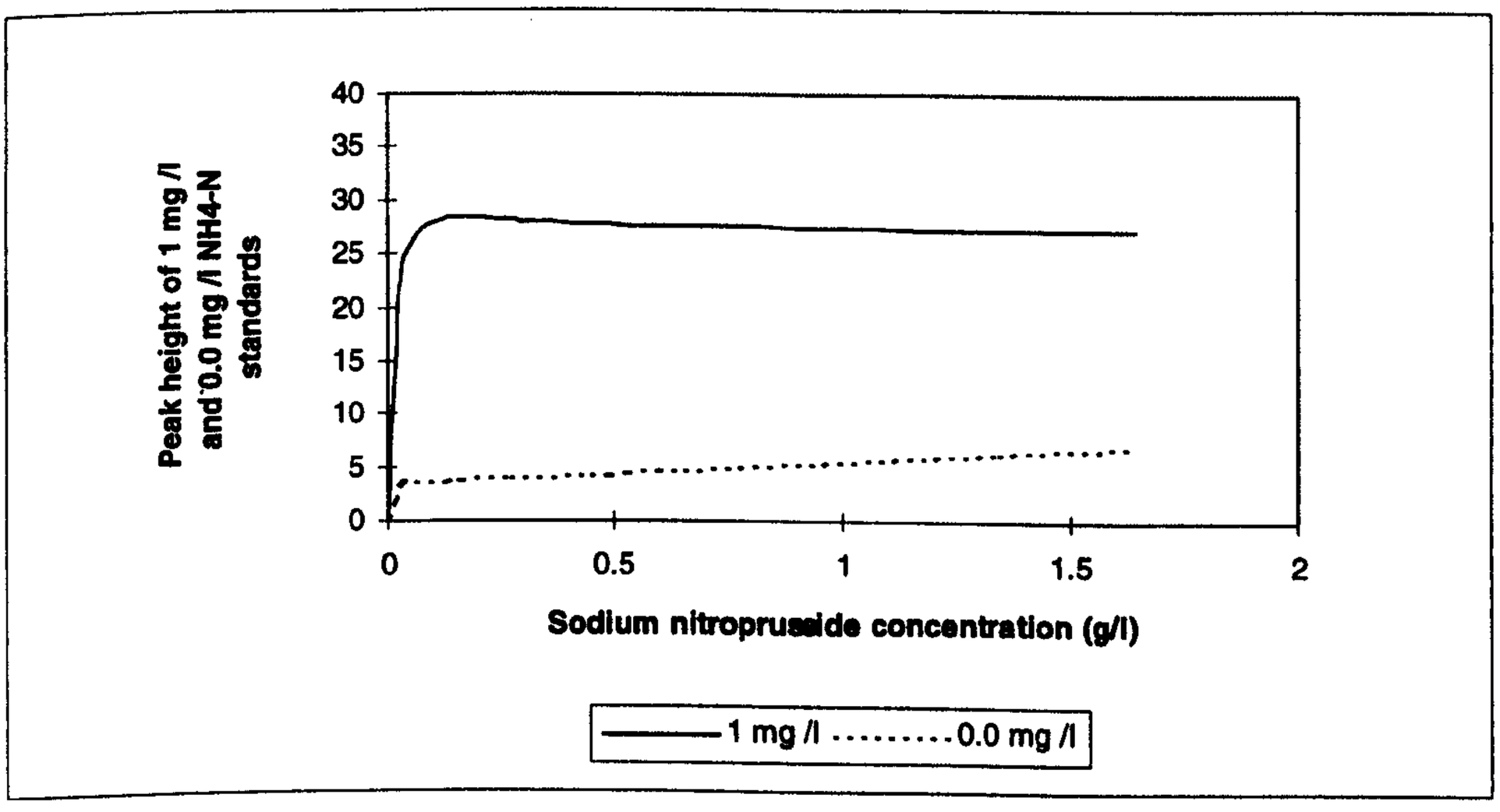


Figure 3.10. : Effect of sodium nitroprusside concentration on the sensitivity of the standard ammonium-N method using the gradient method.

From the results presented in Figure 3.10., the concentration of sodium nitroprusside which gave the best peak height (response) is ranging from 0.15 g /l which is equal to 0.05 g /l in the final solution to 1.6 g /l which is equal to 0.57 g /l in the final solution. The selected concentration of sodium nitroprusside which was chosen is 0.5 g /l which is equal to 0.18 g /l in the final solution.

A similar result was obtained by Harwood and Huyser (1970) who recommended the optimum concentration of sodium nitroprusside to be 0.2 g /l in the final solution.

Other authors suggested different concentrations of sodium nitroprusside as shown in Table 3.5.

Table 3.5. : Sodium nitroprusside concentrations of different authors for the sodium nitroprusside catalysed phenol-hypochlorite method.

Author	Sodium nitroprusside concentration* (g /l)
Brown (1973)	0.04
Berg and Abdullah (1977)	0.23
Mantoura and Woodward (1983)	0.06
Tel and Jansen (1992)	0.08
Technicon Autoanalyzer (Industrial method #624-81W GT)	0.09

* Sodium nitroprusside concentration is in g sodium nitroprusside /l in the final solution.

Comparison of the gradient concentration method with the individual concentration method shows that the gradient method does not give the precise estimate and this may be due to that the gradient peak is not linear in terms of the concentration as well as there is shifting in the base line.

3.1.3.1.6. OPTIMISATION OF SODIUM HYPOCHLORITE CONCENTRATION

(a)- INDIVIDUAL CONCENTRATION METHOD :

The effect of different concentrations of sodium hypochlorite on the sensitivity of the standard ammonium method is illustrated in Figure 3.11.

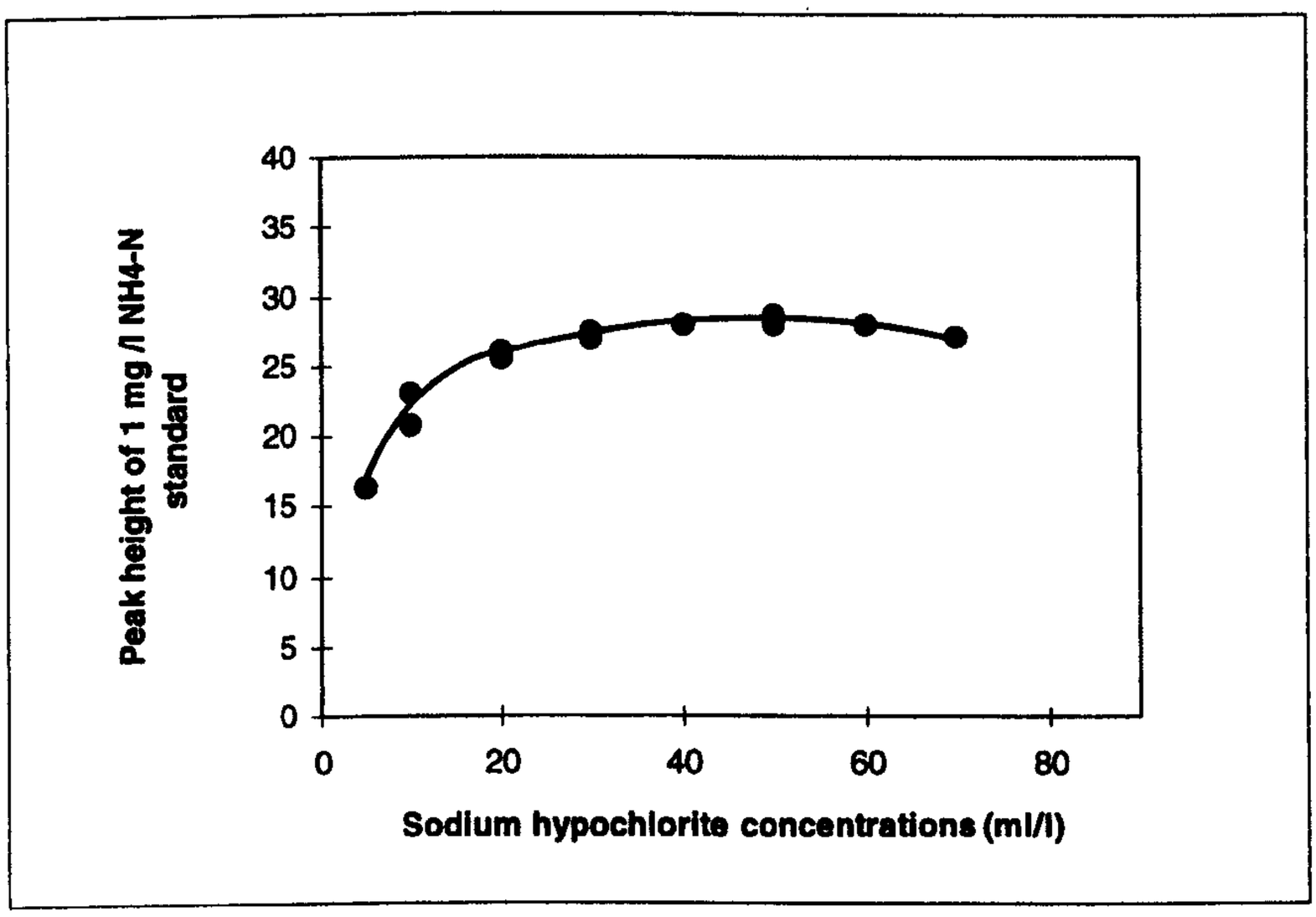


Figure 3.11. : Effect of different concentrations of sodium hypochlorite on the sensitivity of the standard ammonium-N method.

From the results obtained, it is recommended that the optimum concentration of sodium hypochlorite is 50 ml /l, which is equal to 4.76 ml /l in the final solution of the system.

(b)- <u>GRADIENT CONCENTRATION METHOD :</u>

Figure 3.12. shows a graph drawn between the peak height of a 1.0 mg /l NH_4-N standard solution and run time (minutes). Figure 3.13. shows a graph drawn between 1.0 mg /l and 0.0 mg /l NH_4-N standard solutions and the concentration (g /l) of sodium hypochlorite. The concentration of sodium hypochlorite was calculated using the equation described in section 3.1.2.1.2. The base line increases with increasing sodium hypochlorite concentration, therefore, the values for 1.0 mg /l NH_4-N have been corrected by the subtraction of the baseline value.

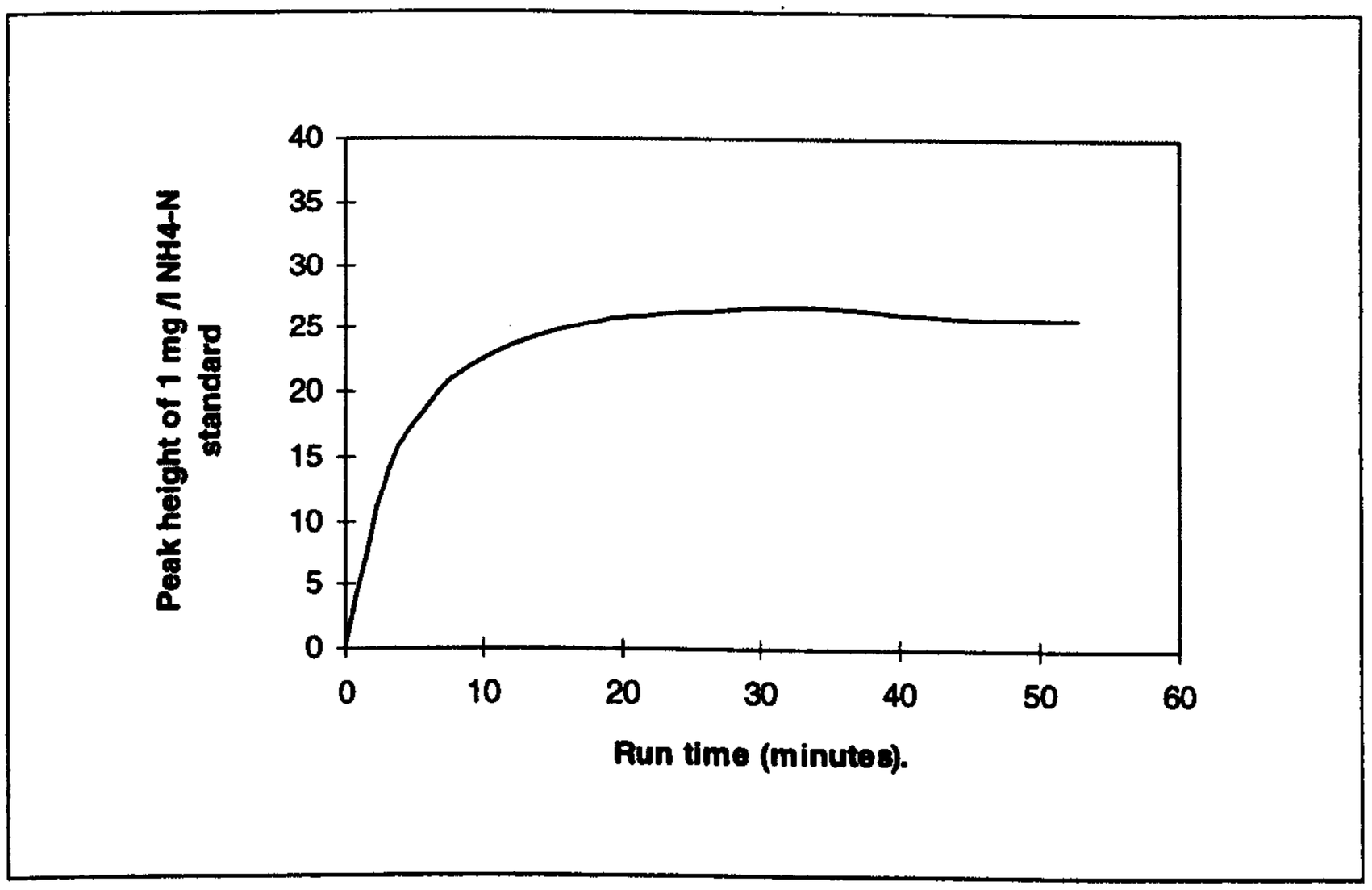


Figure 3.12. : Effect of sodium hypochlorite concentration on the sensitivity of the standard ammonium-N method using the gradient method.

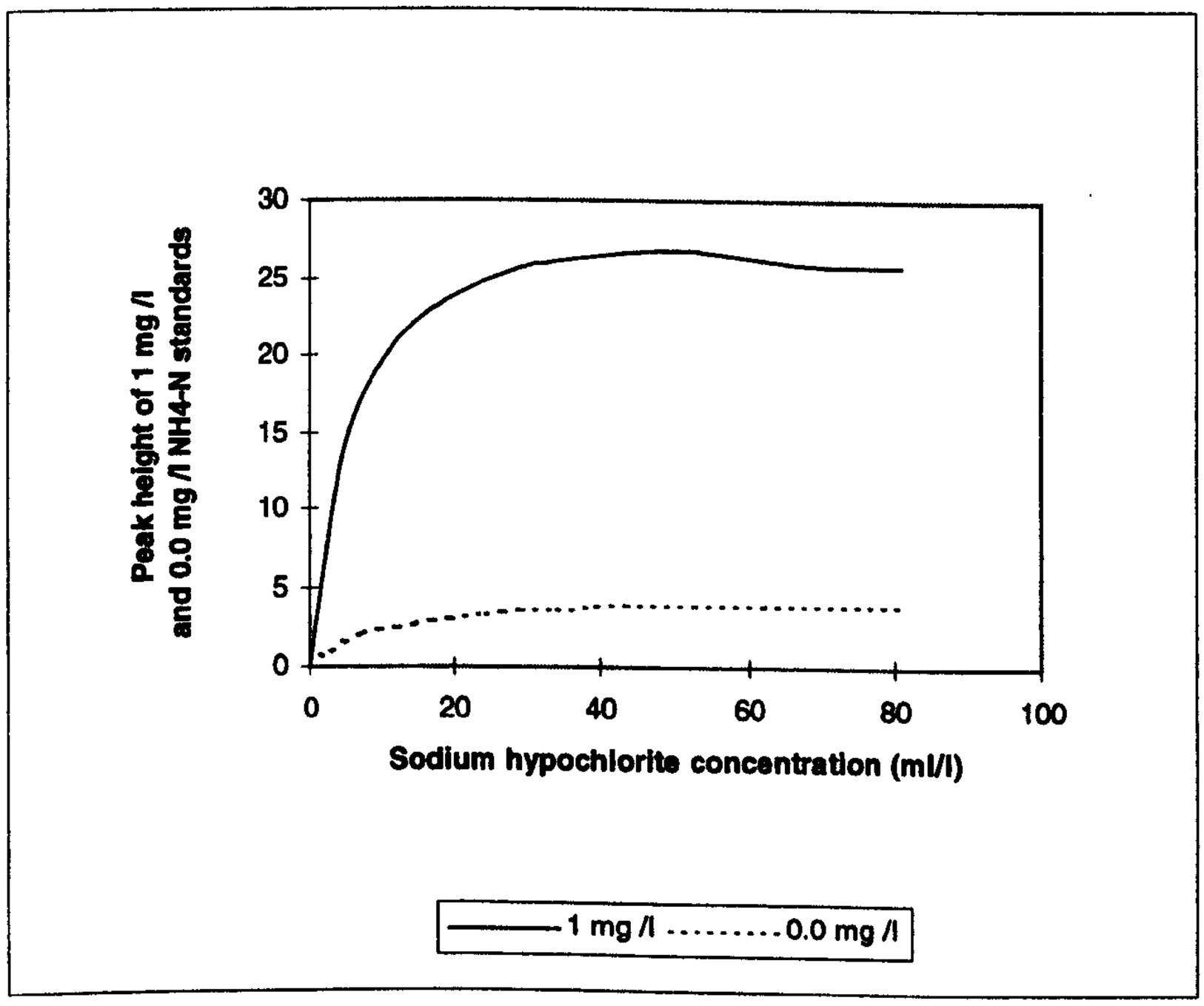


Figure 3.13. : Effect of sodium hypochlorite concentration on the sensitivity of the standard ammonium-N method using the gradient method.

From the results obtained in Figures 3.12. and 3.13., the selected concentration of sodium hypochlorite which gave the best peak height (response) for 1.0

mg /l NH4-N standard solution is 50 ml sodium hypochlorite /l which is equal to 4.76 ml /l in the final solution.

Similar results were obtained by Khan (1994) who studied the effect of different concentrations of hypochlorite on the interference caused by the amino acids in the phenol method. He found that all the amino acids tested caused negative interference but the negative interferences were much reduced with the use of 50 ml hypochlorite /l.

Searle (1984) also reported that negative interferences through the reactions between amino acids and the hypochlorite source occurs with very low hypochlorite concentrations.

Harwood and Huyser (1970) recommended the optimum concentration of sodium hypochlorite to be 0.5 ml /l in the final solution.

Other authors suggested different concentrations of sodium hypochlorite as shown in Table 3.6.

Table 3.6. : Sodium hypochlorite concentrations of different authors for the sodium nitroprusside catalysed phenol-hypochlorite method.

Author	Sodium hypochlorite concentration* (ml /l)
Brown (1973)	3.83
Tel and Jansen (1992)	16.21
Technicon Autoanalyzer (Industrial method #624-81W GT)	11.20

* Sodium hypochlorite concentration is in ml sodium hypochlorite /l in the final solution.

3.1.3.1.7. ABSORPTION SPECTRUM OF THE COLOURED PRODUCT

Figure 3.14. shows the absorbance of the coloured product. It was observed from the figure that the maximum absorbance of the coloured product is at wavelength equal to 640 - 650 nm. Therefore, it is recommended that 650 nm filter can be used effectively providing high sensitivity.

Other authors suggested different wavelengths of filter as shown in Table 3.7.

Table 3.7. : Wavelength of filter of different authors for the sodium nitroprusside catalysed phenol-hypochlorite method.

Author	Wavelength of filter (nm)
Harwood and Huyser (1970)	625
Brown (1973)	625
Berg and Abdullah (1977)	625
Mantoura and Woodward (1983)	630
Tel and Jansen (1992)	660
Technicon Autoanalyzer (Industrial method #624-81W GT)	630

Krom (1980) used a salicylate method and demonstrated that the λ_{max} . was 650 - 660 nm for all combinations of reactants and reaction conditions. There were however, slight differences in the spectrum produced using aquopentacyanoferrate (II) [AqF] and sodium nitroprusside catalysts and with sodium nitroprusside between development of the colour at 35 °C in room lightening compared with at 20 °C in the dark.

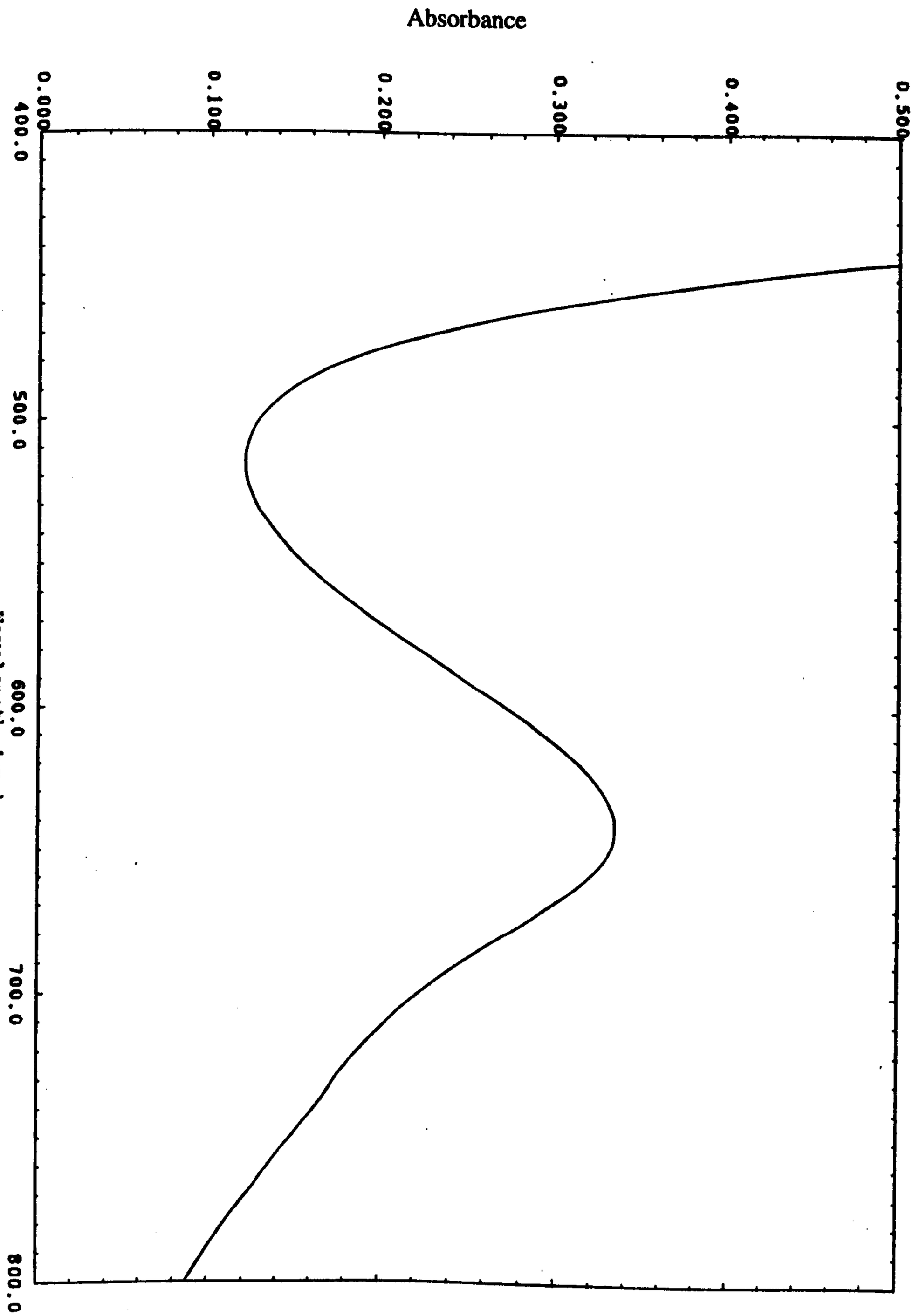


Figure 3.14. : Absorption spectrum of the indophenol product.

3.1.3.1.8. OPTIMISATION OF THE TEMPERATURE OF THE MANIFOLD WATER BATH

The effect of different temperatures of the manifold water bath on the sensitivity of the standard ammonium method is presented in Figure 3.15. The optimum temperature is 30 °C, but from the results obtained there is little difference in the peak height over the range 30 - 45 °C. As the water bath is normally operated at 37 °C for the other determinations, it was decided to continue to use 37 °C for the ammonium-N method.

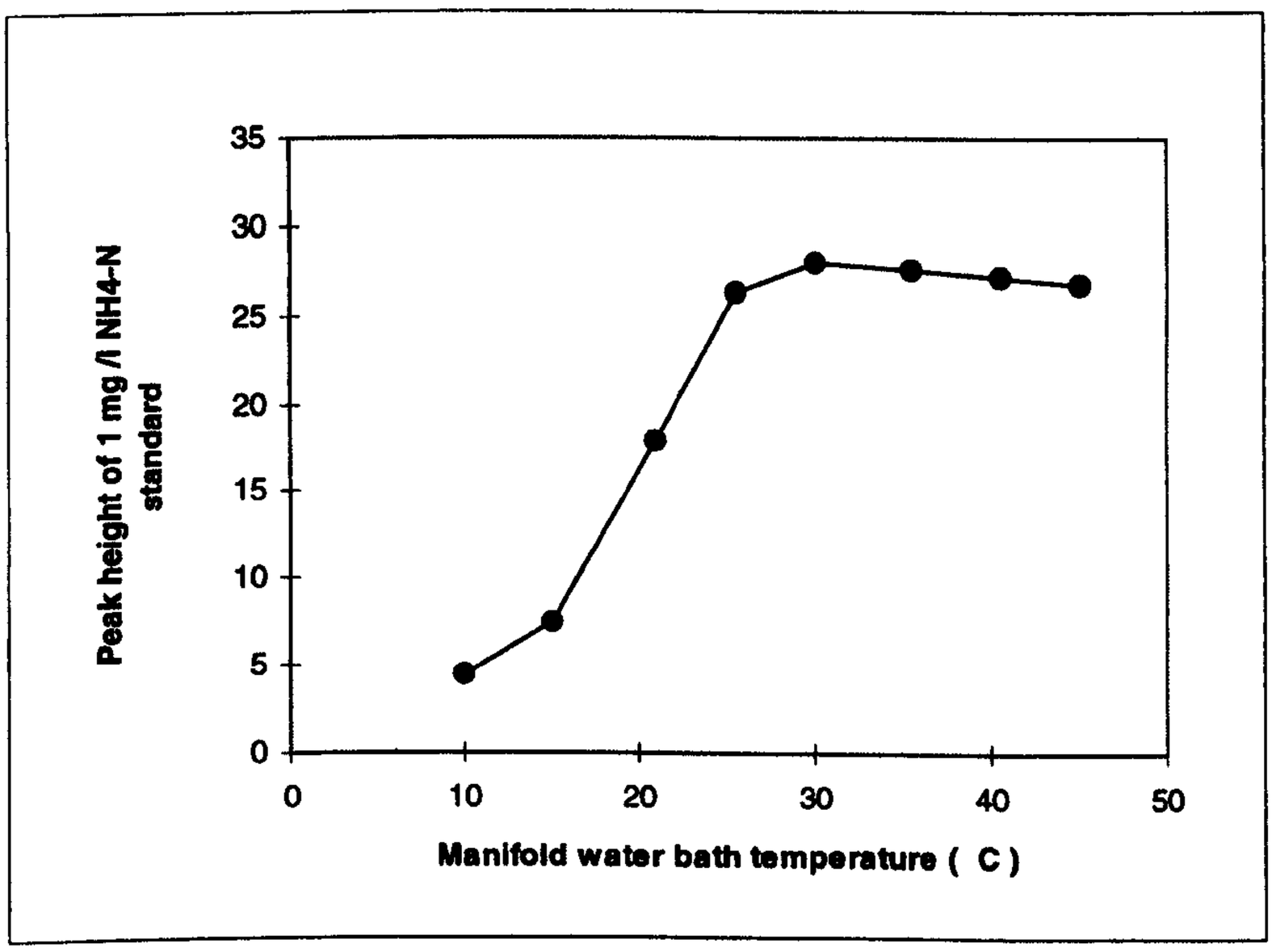


Figure 3.15. : Effect of different temperatures of the manifold water bath on the sensitivity of the standard ammonium-N method.

Other authors suggested different temperatures for the manifold water bath as shown in Table 3.8.

Table 3.8. : Manifold water bath temperature of different authors for the sodium nitroprusside catalysed phenol-hypochlorite method.

Author	Manifold water bath temperature (°C)
Brown (1973)	37.0
Berg and Abdullah (1977)	40.0
Mantoura and Woodward (1983)	50.0
Tel and Jansen (1992)	37.0
Technicon Autoanalyzer (Industrial method #624-81W GT)	50.0

Doyle and Schimel (1996) used the salicylate method and concluded that raising the temperature from 30 to 60 °C doubled the indophenol absorbance. Although the indophenol colour is less stable at high temperatures (Searle, 1984), high temperature accelerates colour development which Doyle and Schimel considered to be useful for the rapid automated analysis.

Having made these optimisations to the standard ammonium-N method, the final method is shown in section 3.1.3.2.

3.1.3.2. FINAL METHOD FOR THE DETERMINATION OF AMMONIUM

REAGENTS :

Analar grade reagents and nitrogen free deionised water were used throughout. These reagents were prepared in a fume cupboard. A fume cupboard was cleared and a 2 figure top pan balance was set up. Care was taken to ensure that the balance was properly locked before attempting to move it. The fan was switched off while actual weighing was in progress but otherwise was kept running. Any spills were cleared immediately. When finished, the balance was wiped down and returned to the balance bench. Finally, the fume cupboard was wiped down.

(a)- Alkaline Phenol :

****** Phenol is highly toxic and caustic. Preparation of this reagent should be carried out in the fume cupboard. Ensure that you are familiar with the MSDS sheet and precautions for handling phenol. *****

25 g of sodium hydroxide was dissolved in approximately 900 ml deionised water in a 1 litre dark glass bottle and then the solution was cooled to room temperature. Working in a fume cupboard, 45 g phenol was weighed very carefully into a 1 litre beaker. Approximately 500 ml sodium hydroxide solution was added and the contents were stirred carefully with a glass rod to dissolve the phenol. Further sodium hydroxide solution was added if necessary. The solution was returned to the bottle and degassed in the ultrasonic bath for 10 minutes. The volume was made to 1 litre with degassed deionised water and the contents were mixed gently. A plastic stopper was used not a glass one.

(b)- Complexing Reagent :

***** Sodium nitroprusside is highly toxic. This reagent should be prepared in a fume cupboard. ******

50 g potassium sodium tartrate and 50 g sodium citrate were dissolved in approximately 900 ml deionised water in a 1 litre bottle and degassed. 0.5 g sodium

nitroprusside was weighed carefully into a 100 ml beaker, 50 ml degassed deionised water was added and the contents were stirred gently using a magnetic stirrer. The resulting solution was added to the citrate tartrate solution and degassed in the ultrasonic bath for 10 minutes. 1.0 ml of 15% Brij-35 solution was added and the volume was made to 1 litre with degassed deionised water and the contents were mixed gently.

(c)- Sodium Hypochlorite Solution (Approximately 0.5 %) :

***** Sodium hypochlorite is a caustic bleaching agent. It will produce chlorine gas with acids. This reagent should be prepared in a fume cupboard. *****

Using a measuring cylinder 50 ml of sodium hypochlorite solution (12 % w/v available chlorine) was added to 1 litre degassed deionised water and the contents were mixed gently.

(d)- Ammonium nitrogen standard stock solution (1000 mg /l) :

Ammonium sulphate was dried at 105 °C for 1 hour and cooled in a desiccator for 30 minutes. 4.717 g of dry ammonium sulphate was dissolved in approximately 900 ml deionised water in 1 litre volumetric flask and the volume was made to 1 litre with deionised water. The solution was stored at 2 °C. Working standards were prepared by the dilution in the appropriate extracting solutions.

PROCEDURE :

The manifold (Figure 3.16.) can be used for the determination of ammonium in water, 2.0 M and 1.0 M potassium chloride, 0.5 M potassium sulphate and several other extractants. The flow rate of the final system is shown in Table 3.9.

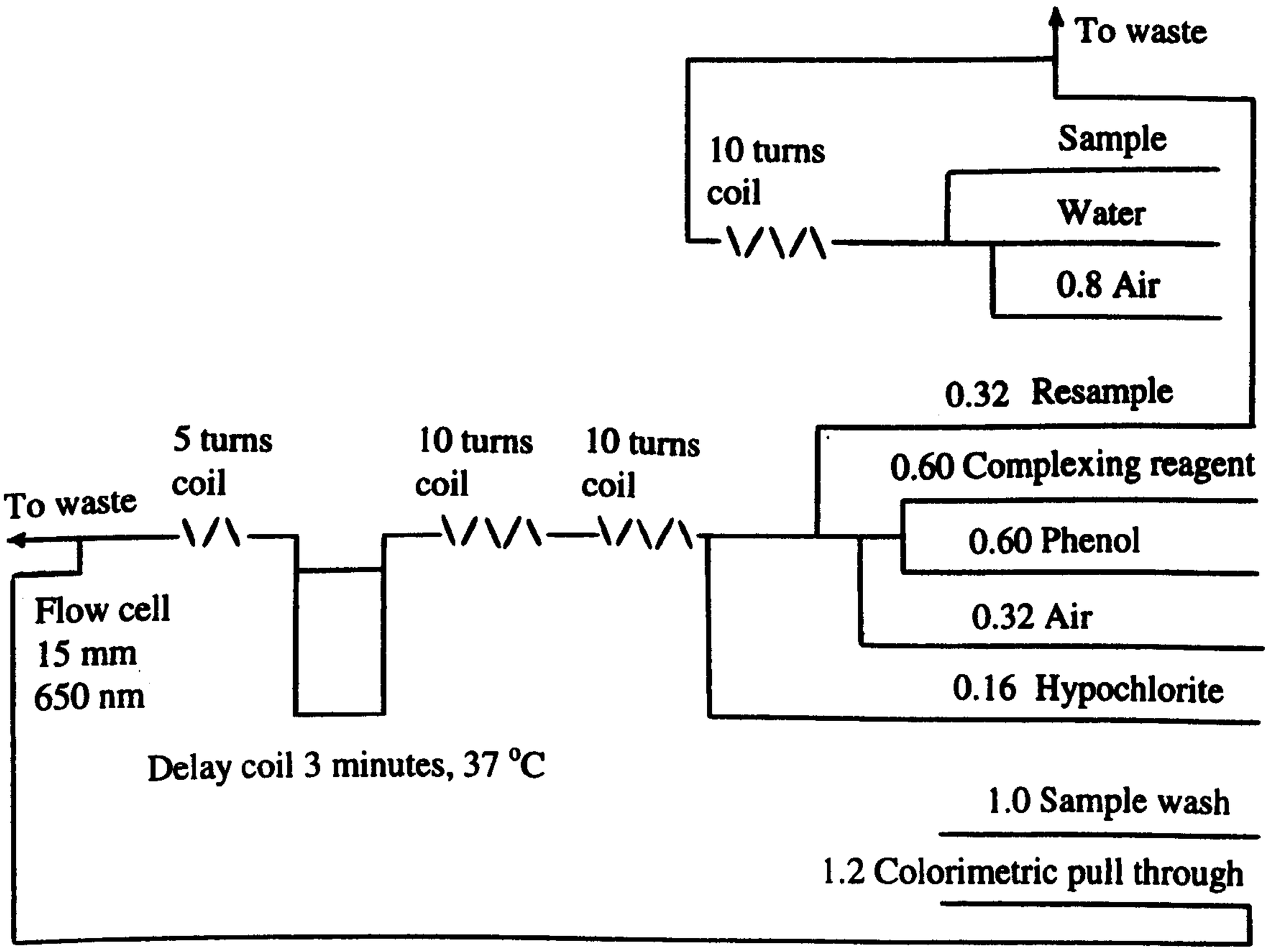


Figure 3.16. : Technicon Autoanalyzer II manifold of the final method for the determination of NH_4-N.

Table 3.9. : Reagent flow rate of the optimised method for the NH_4-N determination.

Tube	Flow rate (ml /min.)
Sample	0.32
Hypochlorite	0.16
Complexing Reagent	0.60
Alkaline Phenol	0.60
Air	0.32
Pull Through	1.20

The solutions can be analysed using the manifold shown in Figure 3.16. along with standard solutions, blanks and zeros. The samples can be run at the rate of 50 per

hour but where dilution is required the sampling rate is reduced to 40 per hour. The colour is developed in the manifold water bath at 37 °C. The colour intensity is measured at 650 nm. The air is cleaned of atmospheric ammonia by bubbling through 5.0 % HCl solution. The calibration graph for ammonium is linear from 0.0 to 5.0 mg NH_4-N /l. Samples with ammonium - nitrogen concentrations higher than 5.0 mg /l are diluted into the range 0.0 to 5.0 mg /l using an inbuilt diluter. Appropriate dilution is carried out for concentrations above 5.0 mg /l. The selected pH for this system is 12.1.

3.1.3.3. ASSESSMENT OF THE PRECISION OF THE OPTIMISED METHOD FOR THE AMMONIUM DETERMINATION

The precision of the optimised method was evaluated by analysing 20 samples of 1.0 and 5.0 mg /l NH_4-N standard solutions using the Autoanalyzer sampler at 40 samples per hour.

The standard deviation (STDEV) and relative standard deviation (RSD %) values are presented in Table 3.10. The results are very reproducible as shown by the low RSD % and are considered acceptable since they are less than 0.5 %.

Table 3.10. : The precision of the optimised method for the ammonium determination.

Standard concentration	Sampling rate	STDEV	RSD %
1.0 mg /l NH_4-N	40 samples per hour	1.190	0.12
5.0 mg /l NH_4-N	40 samples per hour	4.170	0.08

Mazumder (1992) obtained an RSD % as good as 0.18 to 0.40 % for the unoptimised standard method (section 2.11.1.1.) using 10 replicates of 1.0 mg /l NH_4-N standard solution.

Khan (1994) investigated the precision of the unoptimised standard method with the inclusion of a gas phase dialyser and obtained an RSD % of 0.66 % for 1.0 mg /l NH_4-N standard solution.

Comparison of the optimised method with those of other authors shows that it has very good RSD % values and is suitable for the analysis of ammonium-N in soil and water.

3.2. DETERMINATION OF LOW LEVELS OF AMMONIUM (< 0.1 mg N / l) IN GROUNDWATER

3.2.1. INTRODUCTION

In uncontaminated rivers, streams, groundwater and lakes, the ammonium concentration is very low. In general, ammonia and ammonium compounds occur in relatively small quantities in unpolluted waters, on the order of 0.1 mg /l, but organic pollution can result in higher levels. The determination of low levels of ammonium is important for drinking waters, certain surface waters and arctic and glacial ice. At the pH range from 7.4 to 8.5, ammonia is considered to be toxic to diatoms at a level of 1.1 mg /l, and at the same pH range, to fish at a level of 2.5 mg /l (Zadorojny et al., 1973). Accurate measurements below micromolar concentrations are required since ammonium ions are a micronutrient in seawaters. Inadequate sensitivity occurs when methods such as those based on the Nesseler and Berthelot (indophenol blue) reactions are used (Schulze et al., 1988).

Zadorojny et al. (1973) evaluated a manual phenol - hypochlorite method capable of determining low levels of ammonium-N. They considered it a method well suitable for routine ammonia analyses in fresh, estuarine and marine waters and in wastewaters since no major interferences are caused by electrolytes, amino acids, urea, or variations in salinity. The following shows the range of ammonia determination :

With intensifier :

Using a 10 - cm cuvette : 0.001 to 0.10 mg /l NH_4-N.

Using a 1 - cm cuvette : 0.01 to 1.0 mg /l NH_4-N.

Without intensifier :

Using a 1 - cm cuvette : 1.0 to 50 mg /l NH_4-N.

Krom (1980) noted that when sodium nitroprusside is used for the determination of low levels of ammonia, as in sea or lake waters, variable blank values occur through the light - induced production of an absorbing species with a spectrum similar to the blue indophenol dye produced from ammonia.

Hara et al. (1988) developed a simple concentrator based on a microporous poly (tetrafluoroethylene) membrane and polymer nets and combined with an ammonia -

selective gas electrode to construct a continuous flow determination system for low concentrations of ammonium ions in water. A ten - fold increase in the concentration of ammonium ions as well as extension in the linear response limit of an ammonia - selective gas electrode from 30 to about 3 μg /l were obtained when the gas dialysis concentrator was used. The system was used in the determination of residual concentrations of ammonium ions in water purified by distillation and/or de - ionisation and in natural water analysis.

Schulze et al. (1988) investigated several flow - injection methods for their suitability for determining submicromolar levels of ammonium ion on a routine basis. They found that the Nesseler and the Berthelot reactions are not sufficiently sensitive but the gas - diffusion separation is more satisfactory. As the optimised gas - diffusion conditions was used, spectrophotometric detection with an acid - base indicator or the application of a liquid - membrane ammonium - sensitive electrode gives detection limits around 0.14 μg /l. Problems with baseline stability and long - term drift occurs due to the interference of carbon dioxide in the acid - base indicator method. Contamination due to ammonium present in water and reagents severely limited the enhancement of sensitivity by preconcentration. However, effective preconcentration (20 fold in 1 min.) is obtained when the ion - exchange microcolumn placed in the injection loop of the valve was used. With regard to the optimisation of the flow - injection manifold, the choice of acceptor flow rate was critical. For example, at low flow rates, by decreasing the response time of the electrode, the high sensitivity can be counterbalanced. Despite the high sensitivity obtained by the lowest flow rate, these conditions were unsuitable for most analytical applications due to unstable baseline, poor reproducibility (ca. 10 %) and low sample frequency (15 /h). The use of potentiometric detection with a liquid - membrane ammonium - sensitive electrode gave improved baseline stability and slightly better sensitivity. However, regarding the improved detection limits, the limiting factor is the contamination problem. Under normal laboratory conditions, it is difficult to keep the background level present in aqueous solutions below 0.14 μg /l. Therefore, for submicromolar ammonium determinations, the final choice of method must be guided by particular analytical requirements.

Doval et al. (1997) introduced an update of the Kjeldahl method for the direct determination of dissolved organic nitrogen (DON) in seawater. They reported

that the previous removal of dissolved inorganic nitrogen is carried out as follows : ammonium as NH_3 with NaOH at pH 9.4, and subsequently, nitrate and nitrite as nitric oxide with $FeSO_4$ in acid medium. The sample is then mineralized to ammonium, which is measured with a Technicon Autoanalyzer by the indophenol blue method with a range of 14 - 700 µg /l NH_4-N at 630 nm. Comparison showed that the tested standard compounds gave a range of recovery similar to those obtained by high temperature oxidation techniques. Improvement in precision was obtained by the direct determination of DON using the method described, as shown from the comparison with other methods the seawater samples had standard deviation of ± 2.8 µg /l. The precision of ammonium is ± 0.7 µg /l. Dissolved inorganic nitrogen analysis is not a critical factor for the precision of DON measurements. DON values ranged between 42 and 140 µg /l in several stations in the Northeast Atlantic Ocean.

The objective of this study was to develop a reliable Technicon Autoanalyzer method to determine low levels of ammonium in groundwater (< 0.1 mg /l).

The proposed method is based on the optimised reagent concentrations developed earlier. Using more concentrated reagents with lower flow rates enables a larger sample flow rate to be used whilst maintaining the same reagent concentrations in the final reagent stream. This potentially offered a 3 fold increase in sensitivity compared with the standard method (Section 2.11.1.1.).

3.2.2. MATERIALS AND METHODS

3.2.2.1. METHOD DEVELOPMENT

The proposed method is based on the optimised reagent concentrations developed earlier. Using more concentrated reagents with lower flow rates enables a larger sample flow rate to be used whilst maintaining the same reagent concentrations in the final reagent stream. This potentially offered a 3 fold increase in sensitivity compared with the standard method (section 2.11.1.1.).

The samples can be run at the rate of 40 samples per hour. The colour is developed in the manifold water bath at 37 °C. The colour intensity is measured at 650 nm. The air is cleaned of atmospheric ammonia by bubbling through 5 % HCl solution.

As part of the method developing, the following parameters were tested :

1- Effect of the potassium sodium tartrate and sodium citrate concentrations on the solubility of the high concentration complexing reagent solution.
2- Assessment of the pulse suppresser tubes.
3- Assessment of the sequence of adding reagents.
4- Assessment of the sample line debubbler.
5- Assessment of the precision of the final method.

The development work was primarily concerned with obtaining satisfactory low noise levels and good peak shape. The tests of each modification of the system were carried out as follows :

The start - up procedure was followed and the system was allowed to equilibrate. When the chart tracing reached a stable base line, 1.0 mg /l NH_4-N standard solution was run continuously with STD CAL set at 1.0 to observe the noise level on the peak. When the chart tracing reached a stable base line again, 6 samples of 1.0 mg /l NH_4-N standard solution were analysed using the Autoanalyzer sampler at 40 samples per hour to observe the peak shape and to ensure that a peak with a flat top was obtained.

3.2.2.1.1. EFFECT OF THE POTASSIUM SODIUM TARTRATE AND SODIUM CITRATE CONCENTRATIONS ON THE SOLUBILITY OF THE HIGH CONCENTRATION COMPLEXING REAGENT SOLUTION

Different solutions of potassium sodium tartrate and sodium citrate were prepared to determine the combined solubility. These solutions were prepared as follows :

Solution No.	Potassium Sodium Tartrate (g /l)	Sodium Citrate (g /l)
1	50.0	50.0
2	50.0	75.0
3	50.0	100.0
4	50.0	125.0
5	75.0	50.0
6	75.0	75.0
7	75.0	100.0
8	75.0	125.0
9	100.0	50.0
10	100.0	75.0
11	100.0	100.0
12	100.0	125.0
13	125.0	50.0
14	125.0	75.0
15	125.0	100.0
16	125.0	125.0

All solutions contained 1.0 g sodium nitroprusside and 1.0 ml 15% Brij.-35 solution.

3.2.2.1.2. ASSESSMENT OF THE PULSE SUPPRESSER TUBES

The following pulse suppresser tubes were tested :

1- Long tubing [Internal diameter = 0.38 mm and length = 43 cm].

2- Short tubing [Internal diameter = 0.38 mm and length = 21.5 cm].

3.2.2.1.3. CHOICE OF THE SEQUENCE OF ADDING REAGENTS

The following sequences of adding reagent were tested :

1- Sequence No. 1 :

a- Debubbled sample.

b- Air bubble.

c- Phenol and complexing reagent solutions pre mixed with stream divider.

d- Hypochlorite.

2- Sequence No.2 :

a- Debubbled sample.

b- Air bubble.

c- Phenol, complexing reagent and hypochlorite pre mixed with stream divider.

3- Sequence No. 3 :

a- Phenol, complexing reagent and hypochlorite solutions pre mixed with stream divider.

b- Air bubble.

c- Debubbled sample.

4- Sequence No. 4 :

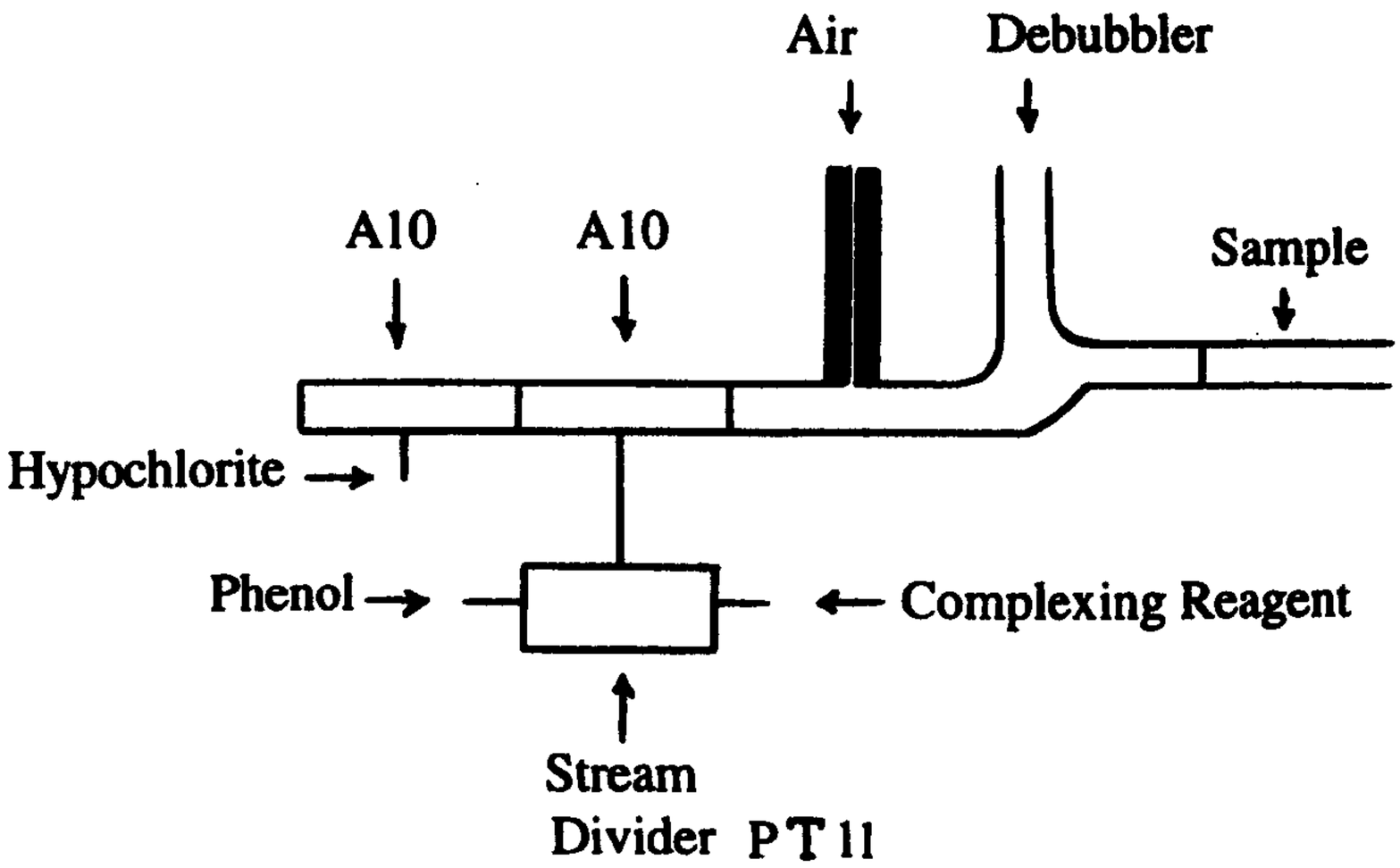
a- Debubbled sample.

b- Air bubble.

c- Phenol solution.

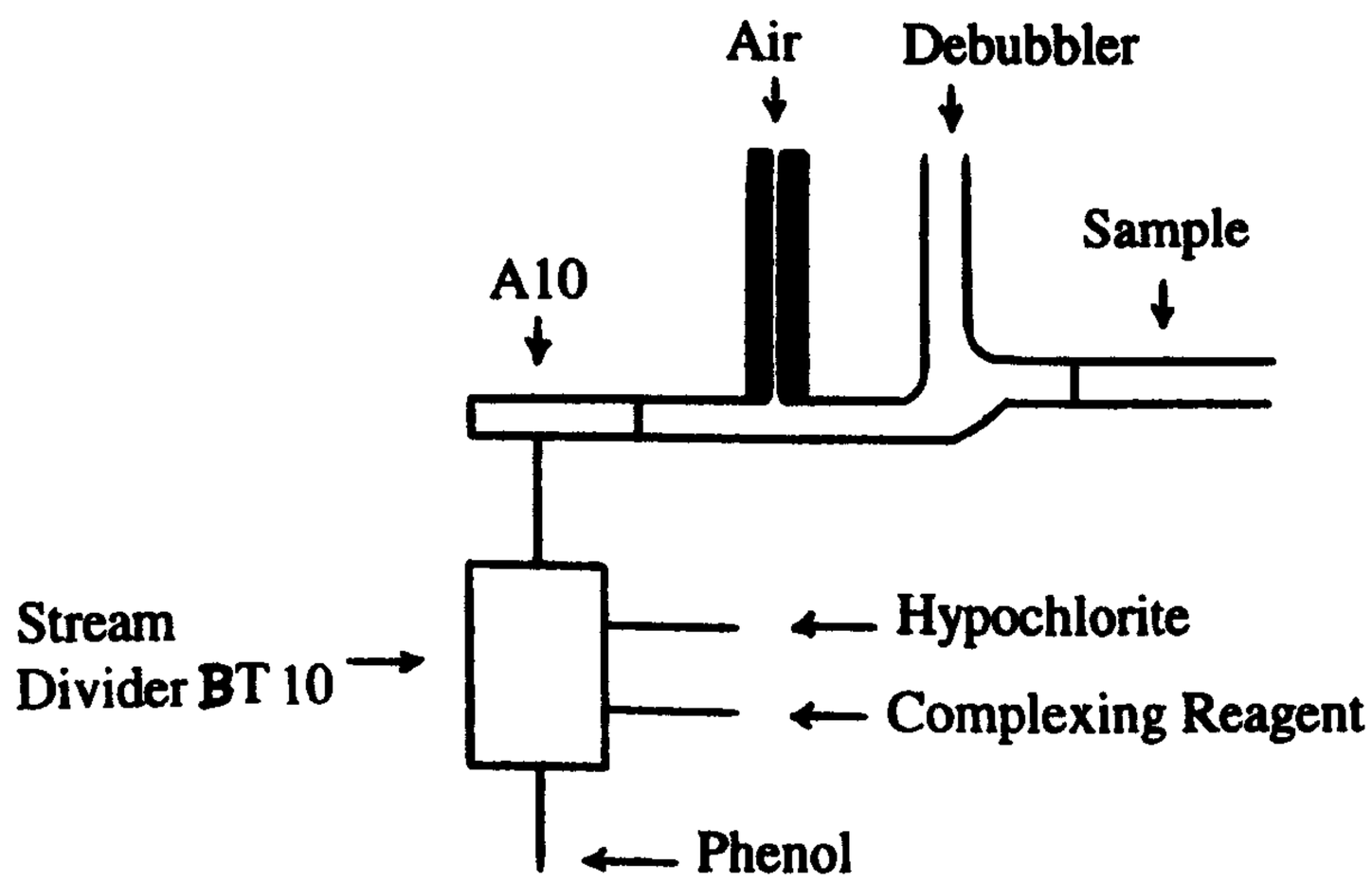
d- Complexing reagent solution.

e- Hypochlorite solution.



Where :
A10 T Junction = 116 B034 01
Stream Divider PT11 = 116 B043 01

Sequence No.1



Where :
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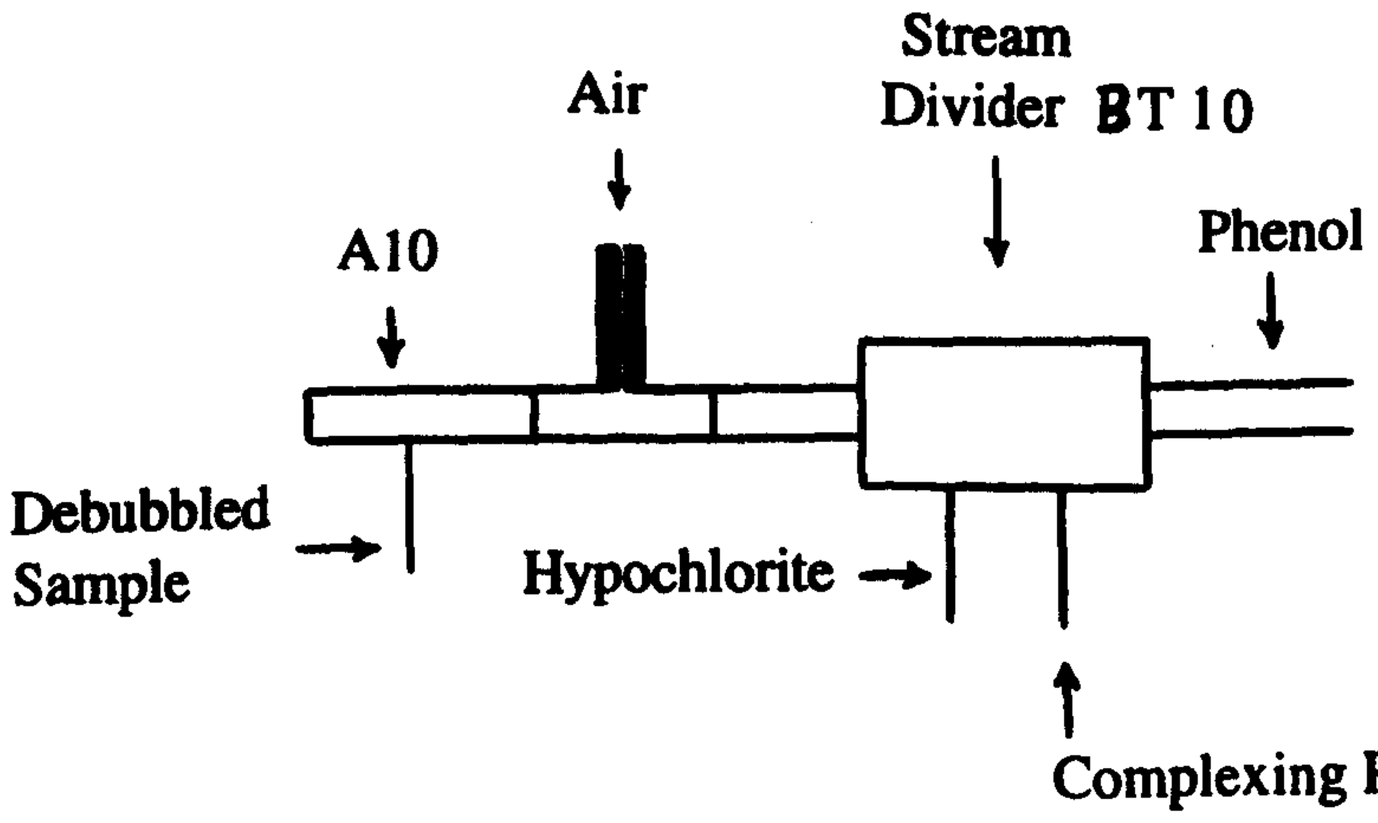
Stream Divider BT10 = 116 B042 01

Sequence No. 2



Where :
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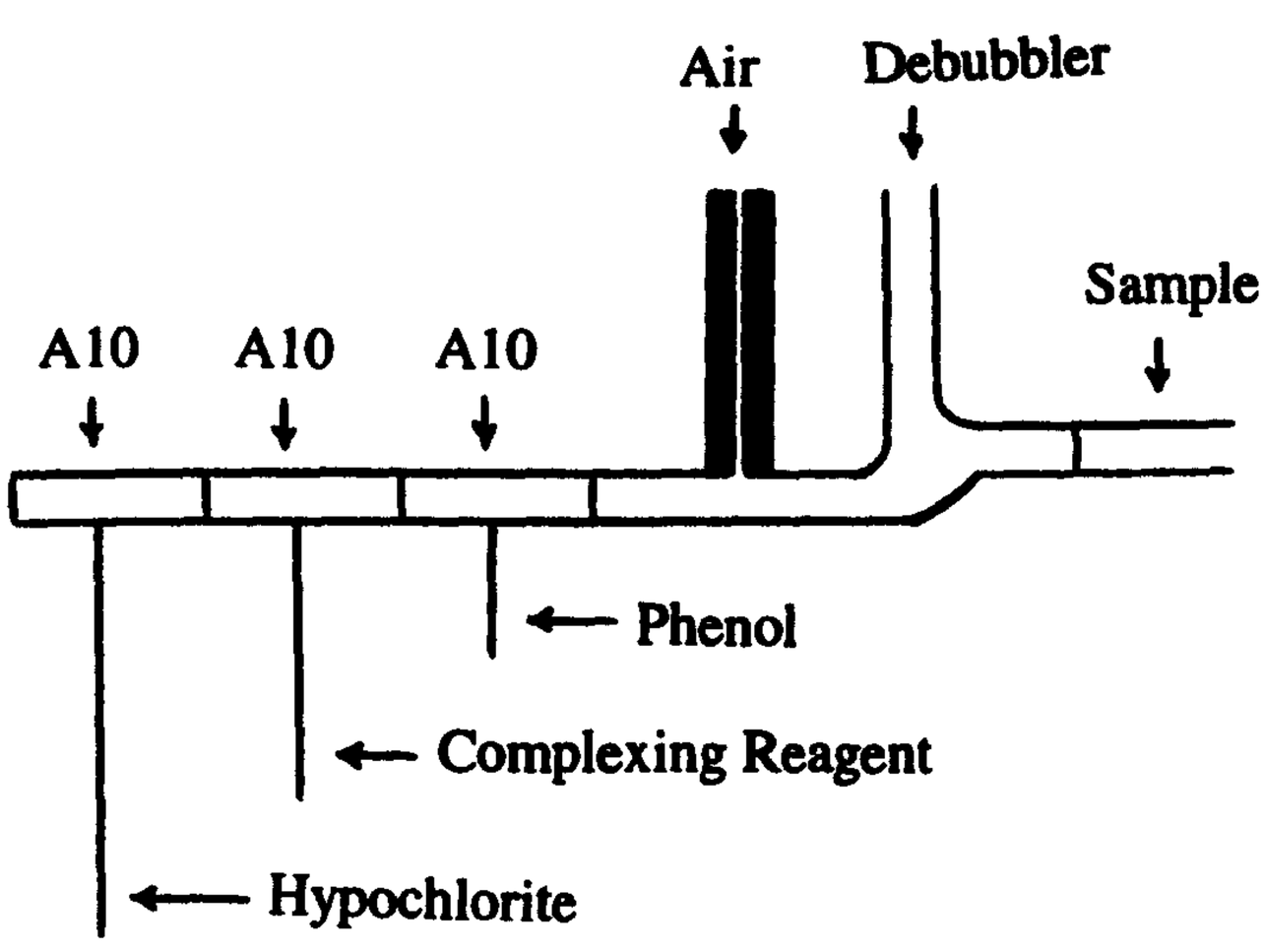
Stream Divider BT10 = 116 B042 01

Sequence No. 3



Where :
A10 T Junction = 116 B034 01

Sequence No. 4

3.2.2.1.4. ASSESSMENT OF THE SAMPLE LINE DEBUBBLERS

The following different sizes and shapes of the sample line debubbler were tested :

1 - T shape debubbler unit No. 1 :

Liquid Stream (ID = 1.8 mm).

Debubbling Stream (ID = 1.8 mm).

Both corners well rounded.

2- T shape debubbler unit No. 2 :

Liquid Stream (ID = 2.3 mm)

Debubbling Stream (ID = 2.3 mm).

Both corners well rounded.

3- T shape debubbler unit No. 3 :

Liquid Stream (ID = 1.8 mm).

Debubbling Stream (ID = 2.3 mm).

Both corners well rounded.

4- T shape debubbler unit No. 4 :

Liquid Stream (ID = 1.8 mm).

Debubbling Stream (ID = 2.3 mm).

Both corners rather squarer.

5- T shape debubbler unit No. 5 :

Liquid Stream (ID = 1.8 mm).

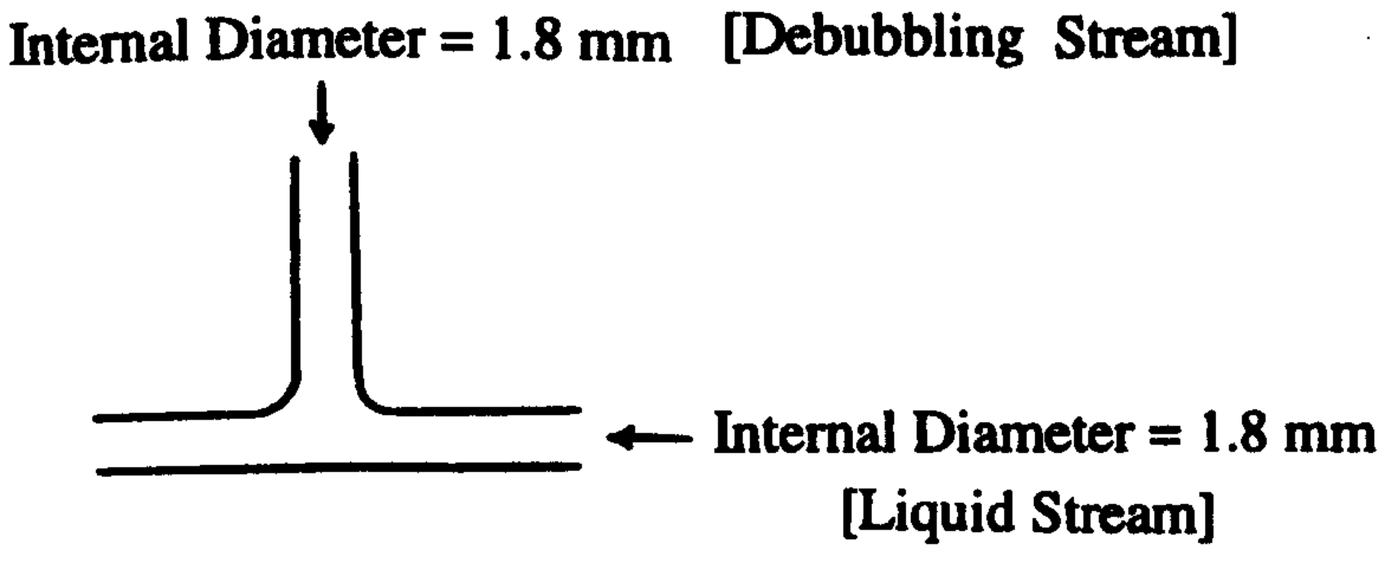
Debubbling Stream (ID = 2.3 mm).

The leading corner rounded, the tailing corner squarer.

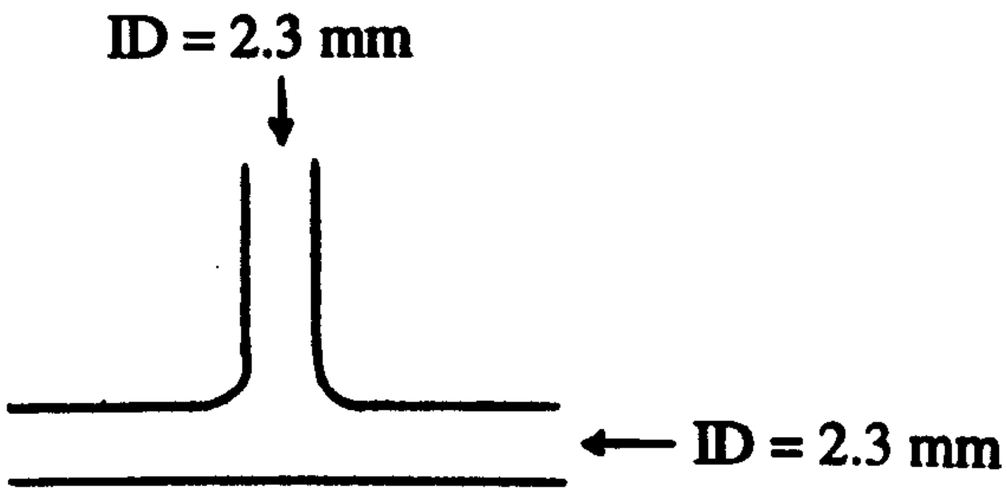
6- Technicon fitting [T 021 G001 01] :

Liquid Stream (ID = 2.0 mm).

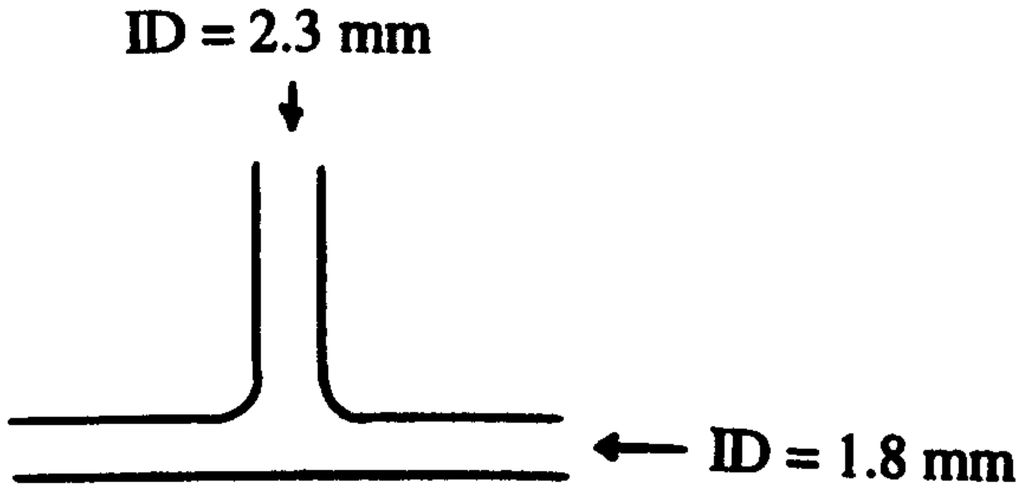
Debubbling Stream (ID = 2.0 mm).



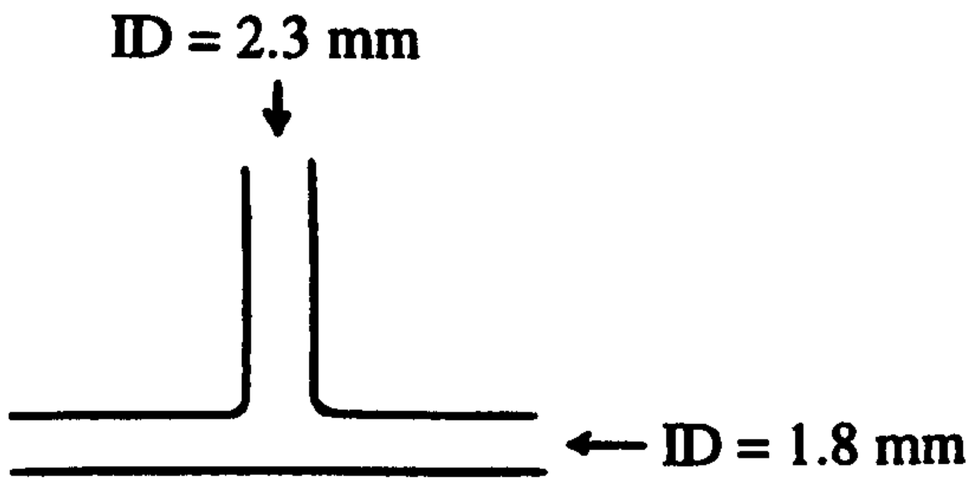
T Shape Debubbler Unit No. 1



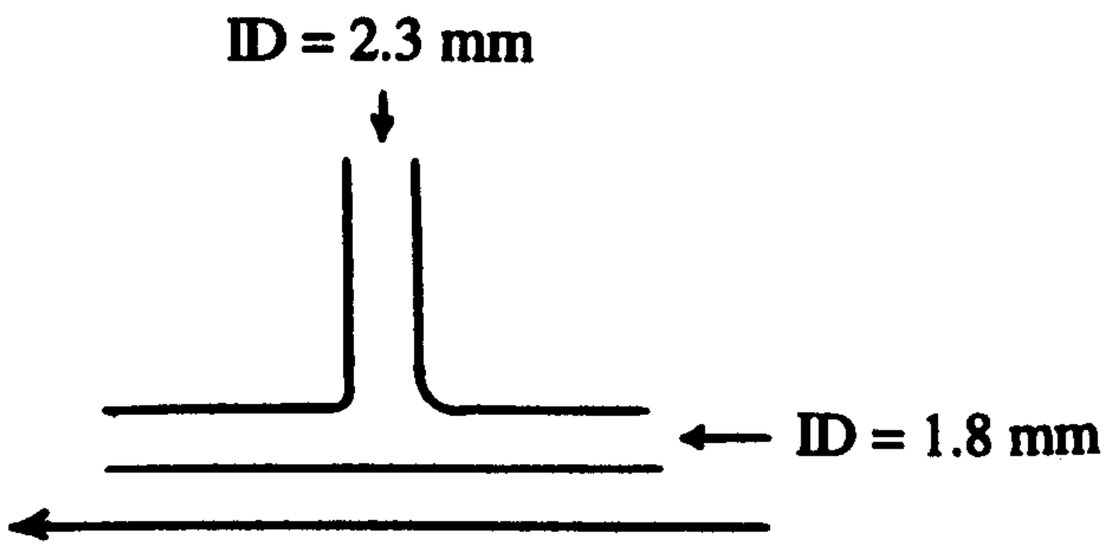
T Shape Debubbler Unit No. 2



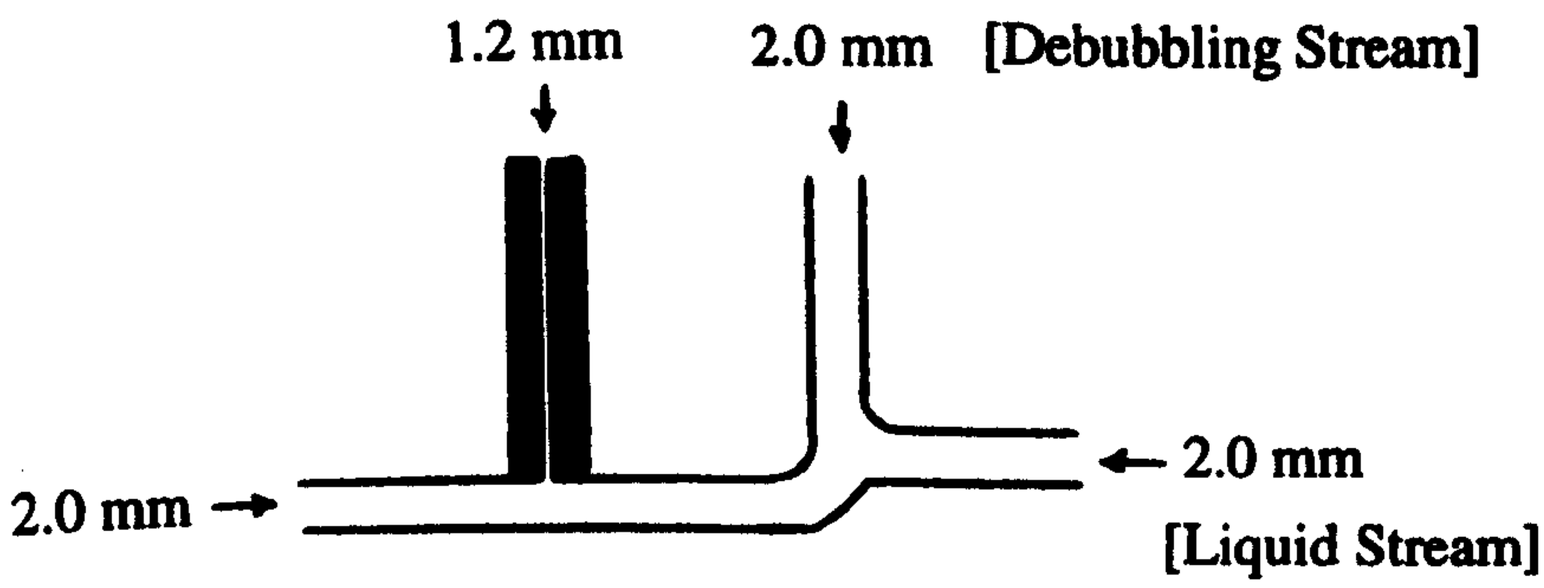
T Shape Debubbler Unit No. 3



T Shape Debubbler Unit No. 4



T Shape Debubbler Unit No. 5



Technicon Fitting [T 021 G001 01]

3.2.2.2. ASSESSMENT OF THE PRECISION OF THE FINAL METHOD

20 samples of 1.0 and 0.1 mg /l NH_4-N standard solutions were analysed separately at 30 and 40 samples per hour.

The statistical analysis for the results was carried out by the Minitab Program (Release 9.2).

3.2.3. RESULTS AND DISCUSSION

The proposed method is based on the optimised reagent concentrations developed earlier. Using more concentrated reagents with lower flow rates enables a larger sample flow rate to be used whilst maintaining the same reagent concentrations in the final reagent stream. This potentially offered a 3 fold increase in sensitivity compared with the standard method (section 2.11.1.1.).

The flow rate and recalculated reagent concentrations of the proposed method are summarised in Table 3.11. The proposed reagent concentrations were obtained by rounding the recalculated reagent concentrations. The schematic diagram of the proposed method is shown in Figure 3.17.

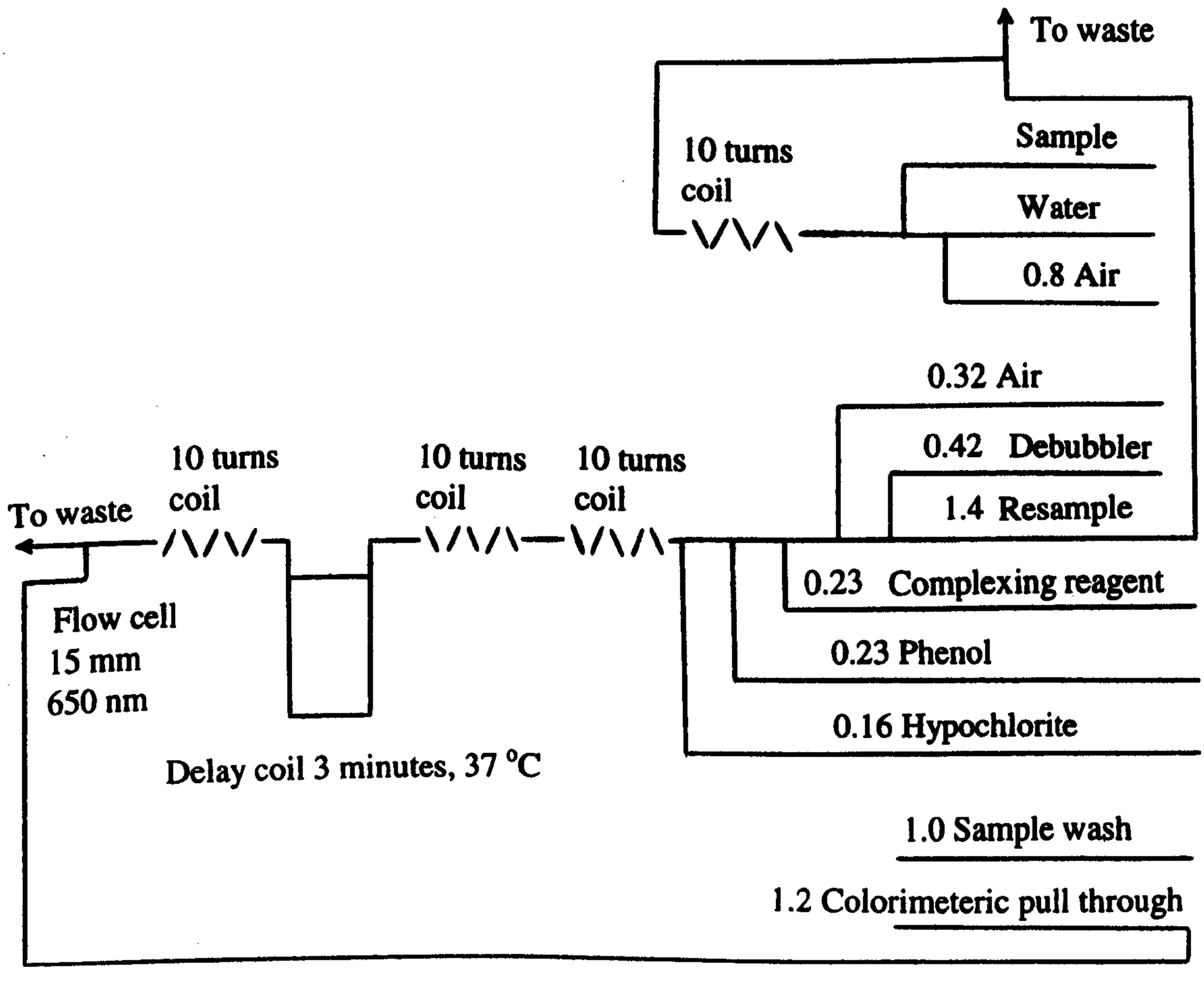


Figure 3.17. : Technicon Autoanalyzer II manifold of the proposed method for the determination of low level of NH_4-N in groundwater.

Table 3.11. : Flow rates, recalculated and proposed reagent concentrations.

Reagent	Flow rate (ml / min.)	Recalculated concentration	Proposed concentration
- Sample*	1.40		
- Sample line debubbler	0.42		
- Sodium Hypochlorite	0.16	47.62 ml /l	50.00 ml /l
-Complexing Reagent :	0.23		
a- Potassium sodium tartrate		124.22 g /l	100.00 g /l
b- Sodium citrate		124.22 g /l	100.00 g /l
c- Sodium nitroprusside		1.24 g /l	1.00 g /l
- Alkaline Phenol :	0.23		
a- Phenol		111.80 g /l	110.00 g /l
b- Sodium Hydroxide		62.11 g /l	60.00 g /l
- Air	0.32		
- Pull Through	1.20		

* Because of high sample flow rate from the tubing connecting the sample probe to the sample pump tubing the sample connecting tube was increased from ID = 0.76 mm to ID = 1.01 mm.

The problems related to developing the system were :

(a) - Preparation of the desired high concentration of a complexing reagent solution proved to be impossible because of insufficient solubility of the potassium sodium tartrate and sodium citrate.

(b) - The injection of the air bubble into the flow using the air bar caused a backwards flow into the debubbler, therefore, a pulse suppresser tube was used in the air to eliminate this problem.

(c) - There is a large inter sample air bubble and this should be removed. Therefore, a sample line debubbler was used to remove the air bubbles from the sample before the reagents were added to the sample. The flow rate of the sample is set to 1.40 ml /min. and the sample line debubbler takes 0.42 ml /min. of the sample to the waste. Therefore, the flow rate of the sample was designed to be 0.98 ml /min.

3.2.3.1. EFFECT OF THE POTASSIUM SODIUM TARTRATE AND SODIUM CITRATE CONCENTRATIONS ON THE SOLUBILITY OF THE HIGH CONCENTRATION COMPLEXING REAGENT SOLUTION

The complexing reagent solution was prepared based on the recalculated concentration (Table 3.11.) but there was a precipitation in the solution. Therefore, a range of complexing reagent solutions of different concentrations of potassium sodium tartrate and sodium citrate were prepared to obtain the best (clear) solution which had no precipitation.

Table 3.12. shows the solubility of each of these solutions of potassium sodium tartrate and sodium citrate. As can be seen from the results, the precipitation occurred in the solution as a result of increasing the concentration of potassium sodium tartrate and sodium citrate. As the complexing reagent solution should contain as high as concentration of potassium sodium tartrate and sodium citrate as possible, it is recommended that the concentration of 100 g potassium sodium tartrate + 100 g sodium citrate /l is used.

Table 3.12. : Effect of the concentrations of potassium sodium tartrate and sodium citrate on the solubility of the high concentration complexing reagent solution.

Reagent	50 g sodium citrate /l	75 g sodium citrate /l	100 g sodium citrate /l	125 g sodium citrate /l
50 g potassium sodium tartrate /l	Clear	Clear	Clear	Clear
75 g potassium sodium tartrate /l	Clear	Clear	Clear	Clear
100 g potassium sodium tartrate /l	Clear	Clear	Clear	Cloudy
125 g potassium sodium tartrate /l	Clear	Clear	Cloudy	Cloudy

From section 3.1.3.1.4., it is clear that all the concentrations of the potassium sodium tartrate and sodium citrate have no effect on the sensitivity of the method, therefore, the reduction in the concentration from 124.22 g /l to 100 g /l would not be expected to have any impact.

3.2.3.2. ASSESSMENT OF THE PULSE SUPPRESSER TUBES

While running the system, the injection of the air bubble into the flow using the air bar caused a backwards flow into the debubbler, therefore, a pulse suppresser tube was used in the air tube to eliminate this problem.

Two lengths (21.5 and 43 cm) of internal diameter = 0.381 mm tubing were tested.

Table 3.13. : Assessment of the pulse suppresser tubes.

Pulse suppresser tube	Peak shape of the individual peaks	Noise level in the individual peaks
Short tubing	Poor (pointed)	Poor
Long tubing	Acceptable	Acceptable

From the results obtained in Table 3.13., it is recommended that the 43 cm pulse suppresser tube is the best one to be used.

3.2.3.3. INFLUENCE OF THE SEQUENCE OF ADDING REAGENTS ON THE PROPOSED METHOD

Different sequences of adding reagents were tested since it is an essential criteria to obtain the best response.

The susceptibility of the reaction to interferences as a result of the hydrolysis of organic nitrogen compounds by the alkaline hypochlorite will be affected by the sequence of adding reagents (Wearne, 1963). The influence of different sequences of adding reagents on the sensitivity of the proposed method is presented in Table 3.14. The assessment was based on the peak shape and the noise level in the individual peaks.

Table 3.14. The influence of the sequence of adding reagents on the sensitivity of the proposed method.

Reagent sequence	Peak shape of the individual peaks	Noise level in the individual peaks
- Sequence No. 1 : a- Sample b- Phenol + Complexing reagent c- Hypochlorite	Acceptable	Acceptable
- Sequence No. 2 : a- Sample b- All reagents pre mixed	Borderline	Borderline
- Sequence No. 3 : a- All reagents pre mixed b- Sample	Poor (pointed)	Borderline
- Sequence No. 4 : a- Sample b- Phenol c- Complexing reagent d- Hypochlorite	Borderline	Borderline

Based on the peak shape and the noise level in the individual peaks, the best sequence of adding reagents is sequence No. 1 : Pre mixed phenol and complexing reagent solutions (0.46 ml /min.) added to the sample line (0.98 ml /min.) followed by the hypochlorite solution (0.16 ml /min.). Adding smaller flows to the larger main flow gave a smooth overall flow and good peak shape.

3.2.3.4. ASSESSMENT OF THE SAMPLE LINE DEBUBBLER

A noise in the signal occurred at the moment the large inter sample air bubble passed through the colorimeter, therefore, a sample line debubbler was tested to remove the air bubbles from the sample.

Assessment of the debubblers was carried out qualitatively based on the peak shape and the noise level in the individual peaks as well as the noise level in the continuous peak.

Tests were made using different sizes and shapes of debubblers to establish a debubbler which gave a good smooth flow.

Table 3.15. illustrates the peak shape and the noise level for each one of these debubblers. It can be seen from the results that there are obvious differences in the peak shape as well as the noise level of the individual peaks. The best peak shape and the minimum noise level in the peak was obtained by using debubbler No. 3 which was well rounded in both corners of the T junction.

Although the debubbler No. 4 gave a good peak shape and low noise level in the individual peaks, debubbler No. 3 gave a much smoother flow.

Table 3.15. : Assessment of the sample line debubblers.

Sample line debubbler	**Noise level in the continuous peak**	**Peak shape of the individual peaks**	**Noise level in the individual peaks**
Technicon fitting	ND*	Acceptable	Acceptable
Debubbler No. 1	Acceptable	Borderline	Borderline
Debubbler No. 2	Acceptable	Borderline	Borderline
Debubbler No. 3	Acceptable	Acceptable	Acceptable
Debubbler No. 4	Acceptable	Acceptable	Acceptable
Debubbler No. 5	Acceptable	Borderline	Borderline

ND* = Not determined

Having made these modifications to the proposed method, the final method is shown in section 3.2.3.5.

3.2.3.5. FINAL METHOD FOR THE DETERMINATION OF LOW LEVELS OF AMMONIUM-N IN GROUNDWATER

REAGENTS :

Analar grade reagents and nitrogen free deionised water were used throughout. These reagents were prepared in a fume cupboard. A fume cupboard was cleared and a 2 figure top pan balance was set up. Care was taken to ensure that the balance was properly locked before attempting to move it. The fan was switched off while actual weighing was in progress but otherwise was kept running. Any spills were cleared up immediately. When finished, the balance was wiped down and returned to the balance bench. Finally, the fume cupboard was wiped down.

(a)- Alkaline Phenol :

***** Phenol is highly toxic and caustic. Preparation of this reagent should be carried out in the fume cupboard. Ensure that you are familiar with the MSDS sheet and precautions for handling phenol. *****

60.0 g of sodium hydroxide was dissolved in approximately 900 ml deionised water in a 1 litre dark glass bottle and the solution was cooled to room temperature. Working in a fume cupboard, 110.0 g phenol was weighed very carefully into a 1 litre beaker. Approximately 500 ml sodium hydroxide solution was added and the contents were stirred carefully with a glass rod to dissolve the phenol. Further sodium hydroxide solution was added if necessary. The solution was returned to the bottle and degassed in the ultrasonic bath for 10 minutes. The volume was made to 1 litre with degassed deionised water and the contents were mixed gently. A plastic stopper was used not a glass one.

(b)- Complexing Reagent :

***** Sodium nitroprusside is highly toxic. This reagent should be prepared in a fume cupboard. *****

100.0 g potassium sodium tartrate and 100.0 g sodium citrate were dissolved in approximately 900 ml deionised water in a 1 litre bottle and degassed for 10 minutes in the ultrasonic bath. 1.0 g sodium nitroprusside was weighed carefully into a 100 ml beaker, 50 ml degassed deionised water was added and the contents were stirred gently using a magnetic stirrer. The resulting solution was added to the citrate tartrate solution and degassed in the ultrasonic bath for 10 minutes. 1 ml of 15% Brij-35 solution was added and the volume was made to 1 litre with degassed deionised water and the contents were mixed gently.

(c)- Sodium Hypochlorite Solution (Approximately 0.5 %) :

***** Sodium hypochlorite is a caustic bleaching agent. It will produce chlorine gas with acids. This reagent should be prepared in a fume cupboard. *****

Using a measuring cylinder 50 ml of sodium hypochlorite solution (12 % w/v available chlorine) was added to 1 litre degassed deionised water and the contents were mixed gently.

(d)- Ammonium nitrogen standard stock solution (1000 mg /l) :

Ammonium sulphate was dried at 105 °C for 1 hour and cooled in a desiccator for 30 minutes. 4.717 g of dry ammonium sulphate was dissolved in approximately 900 ml deionised water in 1 litre volumetric flask and the volume was made to 1 litre with deionised water. The solution was stored at 2 °C. Working standards were prepared by the dilution in the appropriate extracting solutions.

Care should be taking while preparing standards below 0.14 mg /l NH_4-N since they are difficult to be prepared and to handle due to potentially serious contamination problems (Schulze et al., 1988).

PROCEDURE :

The manifold (Figure 3.17.) can be used for the determination of low levels of ammonium-N in the groundwater (< 0.1 mg /l). The flow rate of the final method is presented in Table 3.16.

Table 3.16. : Reagent flow rate of the final method for the determination of low levels of ammonium-N in groundwater.

Tube	Flow rate (ml /min.)
Sample	1.40
Sample connecting tube (ID = 1.01 mm)	
Sample line debubbler	0.42
Hypochlorite	0.16
Complexing Reagent	0.23
Alkaline Phenol	0.23
Air	0.32
Pulse Suppresser	
(Length = 43 cm and ID = 0.38 mm)	
Pull Through	1.20

The solutions can be analysed using the manifold shown in Figure 3.17. along with standard solutions, blanks and zeros. The samples can be run at the rate of 40 samples per hour. The colour is developed in the manifold water bath at 37 °C. The colour intensity is measured at 650 nm. The air is cleaned of atmospheric ammonia by bubbling through 5 % HCl solution. The calibration graph for ammonium is linear from 0.0 to 0.1 mg NH_4-N /l.

3.2.3.6. ASSESSMENT OF THE PRECISION OF THE FINAL METHOD

The precision of the proposed method was evaluated by analysing 20 samples of 1.0 and 0.1 mg /l NH_4-N standard solutions at 30 and 40 samples per hour.

The standard deviation (STDEV) and relative standard deviation (RSD %) values are given in Table 3.17. The results are very reproducible as shown by the low RSD % and are considered acceptable since they are less than 0.5 %.

Table 3.17. : The precision of the final method for the low levels of ammonium-N determination.

Standard concentration	Sampling rate	STDEV	RSD %
1.0 mg /l NH_4-N	40 samples per hour	0.630	0.06
0.1 mg /l NH_4-N	40 samples per hour	0.310	0.31
0.1 mg /l NH_4-N	30 samples per hour	0.354	0.36

Comparison of this low levels ammonium-N method with the optimised method (section 3.1.3.3.) shows that both have very good RSD % values (Tables 3.10. and 3.17.) and are suitable for analysis of low levels of NH_4-N in groundwater.

Mazumder (1992) obtained an RSD % as good as 0.18 to 0.40 % for the unoptimised standard method (section 2.11.1.1.) using 10 replicates of 1.0 mg /l NH_4-N standard solution.

Khan (1994) investigated the precision of the unoptimised standard method with the inclusion of a gas phase dialyser and obtained an RSD % of 0.66 % for 1.0 mg /l NH_4-N standard solution.

3.3. ANALYSIS OF HIGHLY COLOURED GROUNDWATER SAMPLES

3.3.1. INTRODUCTION

Some of the groundwater samples analysed in this work are highly coloured and since the colorimetric analysis is used for the determination of ammonium-N, nitrate-N, nitrite-N and chloride these samples need decolorisation.

There are two approaches in removing the colour, one is the removal by decolorising agents and the other is using a dialysis system.

Different types of charcoal such as activated charcoal (Darco G60 and Norit), bone charcoal, barbecue charcoal and activated coconut shell carbon were used for the removal of the colour. Different types of polymers such as polyclar SB100 [Polyvinylpyrrolidinone insoluble (PVPP)], polyclar AT [Polyvinylpyrrolidinone insoluble (PVPP)] and polyvinylpyrrolidone soluble (PVP) were used for the removal of the colour. The most commonly used adsorbent materials for the removal of colour are activated charcoal (Darco G 60) and polyclar SB100 [Polyvinylpyrrolidinone insoluble (PVPP)].

Qadeer et al. (1994) conducted a study on the structure of a commercial activated charcoal using different techniques such as X - ray diffraction, thermal analysis, nitrogen adsorption and mercury porosimetry. They pointed out that the efficiency of the activated charcoal depends on its physical adsorption capacity which itself depends on the porosity and the surface properties of the adsorbent. The chemical nature of the activated charcoal is associated with the intrinsic properties of the parent materials as well as with the manufacturing and the activation methods. The activated charcoal is characterised as amorphous in nature and has micro - crystalline structure as shown in the X - ray diffraction study. The application of the pore size distribution curve and D-R analysis of nitrogen adsorption isotherm refers to the microporous nature of the activated charcoal with surface area 1000 m^2 /g, porosity 75.74 % and pore volume 1.43 cm^3 /g. The thermogravimeteric study of activated charcoal showed that the loss in weight occurred in two distinct regions : one is endothermic (dehydration) and other is

exothermic (combustion). The moisture and ash contents were 11 % and 10 %, respectively.

Lew (1983) developed a decolorisation method based on the combined use of strong anion - exchange resins (Diaion PA308 and Duolite A - 102D) and activated carbon (Darco G60 and Norit) instead of lead compounds for decolorising dilute molasses solutions. He found a close agreement between the polarimetry measurements of filtrates obtained from decolorisation by resin - carbon and those following clarification by lead. In addition, the efficiency of the proposed decolorising reagents was similar to that of lead. However, the filtration of the treated samples with resin and carbon was faster than treatment with lead. The efficiency of Darco G60 in the decolorisation was slightly better than that of Norit carbons. Compared with lead, the less toxic decolorising reagents are readily available commercially and relatively inexpensive.

McGeehan et al. (1989) found that the errors in the colorimetric analysis of boron decreased with increasing the addition of decolorising charcoal. Charcoal is effective in reducing the yellow colour resulting from the hot water extraction of soil, however, care should be taken to avoid negative errors due to boron sorption by charcoal.

Meyer et al. (1992) found that with the exception of vermiculite, barbecue charcoal and rice husks showed the best adsorptive qualities (67 % and 65 %, respectively) as they removed more than 50 % of the colour from the wastewater. The use of barbecue charcoal is economically beneficial since it is a cheap natural product compared to activated carbon, can be regenerated for re - use in the same way as the latter as well as it is readily available.

Samuelson and Wennergren (1977) indicated that compared with other resins, a weakly basic anion exchanger in HSO_3^- form gave a better efficiency than that of macroporous styrene - divinylbenzene polymers, acrylic acid esters and crosslinked polyvinylpyrrolidone soluble (PVP) for colour removal. Although the crosslinked polyvinylpyrrolidone soluble (PVP) which acts as a proton acceptor and leads to hydrogen bonding with phenolic and carboxylic acid groups was the most effective in removing the colour (500 nm) during the first period of the sorption, it was less effective by the time 10 bed volumes had passed through than the HSO_3^- form of a weakly basic anion exchange resin (Duolite A - 4F).

Simpson et al. (1982) found that the removal of the natural colour of wine was greater using decolorising carbon than using polyvinylpyrrolidone (PVPP). The carbon treated wines had a reduction in optical density at 400 nm of approximately 30 % which was greater than that for the PVPP treated wines.

Mattick and Rice (1981) indicated that compared to polyclar AT (PVPP), charcoal (Darco KB) can cause variations in the tartrate concentration of the wine due to the previous treatment of the charcoal. Moreover, the decolorisation method by the charcoal is more time consuming.

The inclusion of a dialyser in the manifold of automated colorimetric analysis systems has many advantages such as the removal of interferences like suspended solids and unwanted macromolecules (for example protein) and to make an automated dilution step (van Staden, 1995). In continuous flow analysis, the dialysis system consists of 2 flow streams, the donor stream (sample) and the acceptor stream, which are separated by a semipermeable membrane. There are two different systems of the dialysis, one is the liquid or aqueous phase dialysis and the other is gas phase dialysis. The liquid or aqueous phase is the dialysis system where the ions pass through a wettable membrane (C - membrane) which means the movement of ions is through a liquid film in the membrane to reach a receptor stream. Thompson and Blankley (1984) used this dialysis system for the determination of nitrate - N and nitrite - N. In addition, many authors used this dialysis system for the determination of chloride (Araujo et al., 1995; and van Staden, 1995).

The gas phase dialysis is the dialysis system in which the NH_4-N is converted to NH_3-N and passes as a gas through a not wettable Teflon membrane. Many authors have used this dialysis system for the determination of ammonium - N (Schulze et al., 1988; Martelli et al., 1995; Gerendas et al., 1995; and Moskvin et al., 1998).

The objective of this study is to develop a method for decolorisation which can cope with highly coloured groundwater samples. This investigation includes the use of the charcoal G60 and polyclar SB100 as decolorising agents. In addition, this study includes the use of liquid dialysis and gas phase dialysis which can be included in the chloride, nitrite, nitrate and ammonium manifold systems of the Technicon Autoanalyzer II system.

3.3.2. MATERIALS AND METHODS

All the decolorisation tests were carried in glass bottles so as to see the solution clearly and to avoid the sticking of the charcoal in scratches in the plastic bottles which could contaminate the bottles.

3.3.2.1. USING CHARCOAL G60 AND POLYCLAR SB100 FOR THE DECOLORISATION OF WATER SAMPLES

This study is concerned with the efficiency of charcoal G60 and polyclar SB100 in the decolorisation of water samples, the presence of impurities in the charcoal G60 and the polyclar SB100, cleaning the charcoal G60 and polyclar SB100 from impurities and the adsorption of NH_4-N and NO_3-N from solution by the cleaned charcoal G60 or polyclar SB100.

3.3.2.1.1. EFFICIENCY OF CHARCOAL G60 AND POLYCLAR SB100 IN THE DECOLORISATION OF WATER SAMPLES

Two decolorising agents, polyclar SB100 and charcoal G60 were used.

MATERIALS :

1- Polyclar SB100 (polyvinylpyrrolidone insoluble) :

Polyclar SB100 was supplied by BDH Co. It is an insoluble cross - linked polyvinylpyrrolidone, useful for purification of plant enzymes by absorbing phenols and tannins from aqueous extracts [BDH Laboratory Supplies Catalogue, 1997]. Polyclar is a registered Trade Mark of GAF Corporation. NR.

2- Charcoal decolorising powder activated (Darco G60) :

Charcoal G60 was supplied by BDH Co. Darco is the registered trade mark of Norit U.K. Ltd. NR. [BDH Laboratory Supplies Catalogue, 1997].

This experiment was carried out on 2 groundwater samples (SW 15 and SW 16) which differed in colour and pH as shown in Table 3.18. These groundwater samples were collected from Nobel Enterprises, Ardeer site, Stevenston Ayrshire, Scotland.

Table 3.18. : Description of well waters No. SW 15 and SW 16.

Well No.	Degree of colour*	pH	Sampling date
SW 15	Medium colour (Slightly coloured)	7.54	24 / 4 / 1996
SW 16	Black colour (Highly coloured)	3.53	11 / 4 / 1996

* The colour of the groundwater samples is not because of the sediments.

PROCEDURE :

0.5, 2.5 and 5.0 g polyclar SB100 and 0.5 and 2.5 g charcoal G60 were weighed into a 50 ml glass bottle to which 25 ml water sample was added. The bottle was shaken for 15 minutes by hand, allowed to stand for 15 minutes, then filtered through Whatman No. 1 filter paper so that the colour of the supernatant could be observed.

In the case of 5.0 g polyclar, the polyclar absorbed all the solution, therefore it was centrifuged for 10 minutes at 4500 RPM using the Centrifuger (MSE - Model : Mistral 2000) and filtered through Whatman No. 1 filter paper so that the supernatant could be observed.

3.3.2.1.2. THE PRESENCE OF IMPURITIES IN CHARCOAL G60 AND POLYCLAR SB100

Charcoal G60 or polyclar SB100 extract solution was prepared to determine the levels of impurities present in it which may contaminate the groundwater sample with the use of charcoal or polyclar as a decolorising agent.

PREPARATION OF CHARCOAL G60 OR POLYCLAR SB100 EXTRACT :

1.0 g charcoal G 60 or polyclar SB100 was weighed into a 60 ml glass bottle. 50 ml deionised water was added to the bottle. The bottle was shaked for 30 minutes in an orbital incubator (Gallenkamp - Model : INR 250) at 25 °C , allowed to stand for 15 minutes, the supernatant was filtered through Whatman No. 1 filter paper. NH_4-N, NO_3-N, NO_2-N, PO_4, Na, and K were determined in the filtrate. Five replicates were prepared.

A 9.0 ml of charcoal or polyclar extract was placed in 10 ml volumetric flask and 1.0 ml of 3 % $SrCl_2.6H_2O$ solution was added. Calcium and magnesium were determined in this solution. Five replicates were prepared.

CHEMICAL ANALYSIS :

Ammonium - N was determined by the Technicon Autoanalyzer II system as described in section 3.1.3.2. using a standard series 0.0 to 1.0 mg /l NH_4-N in deionised water.

Nitrate and nitrite were determined by the Technicon Autoanalyzer II system as described in section 2.11.1.3. Nitrate was determined using a standard series 0.0 to 1.0 mg /l NO_3-N in deionised water. Nitrite was determined using a standard series 0.0 to 1.0 mg /l NO_2-N in deionised water.

Phosphate was determined by the Technicon Autoanalyzer II system as described in section 2.11.2. using a standard series 0.0 to 1.0 mg /l P in deionised water.

Sodium was determined by the Flame photometer as described in section 2.11.4. using a standard series 0.0 to 2.5 mg Na^+ /l in deionised water.

Potassium was determined by the Flame photometer as described in section 2.11.3. using a standard series 0.0 to 5.0 mg K^+ /l in deionsed water.

Calcium was determined by the Atomic Absorption Spectrophotometer as described in section 2.11.5. using a standard series 0.0 to 5.0 mg Ca /l in deionised water and contained 10 % of 3 % $SrCl_2.6H_2O$ solution.

Magnesium was determined by the Atomic Absorption Spectrophotometer as described in section 2.11.6. using a standard series 0.0 to 0.5 mg Mg /l in deionised water and contained 10 % of 3 % $SrCl_2.6H_2O$ solution.

3.3.2.1.3. CLEANING CHARCOAL G60 AND POLYCLAR SB100 FROM IMPURITIES

Charcoal G60 or polyclar SB100 was treated in a cleaning process with different cleaning (extracting) solutions. These cleaning solutions were as follows :

a- Deionised water.

b- 0.1 M KCl solution followed by deionised water as a rinse to remove the rest of the KCl.

c- 0.1 M HCl solution followed by deionised water as a rinse to remove the rest of the HCl.

PROCEDURE :

30 g charcoal G60 or polyclar SB100 was added to a 500 ml glass bottle. 300 ml extracting solution was added. The bottle was shaken for 30 minutes in an orbital incubator at 25 °C, allowed to stand for 15 minutes. A portion of the supernatant was filtered through Whatman No. 1 filter paper [filter paper pre washed with 0.5 M H_2SO_4 solution according to Shah (1988) as described in section 2.2.]. NH_4-N, NO_3-N and NO_2-N were determined in the filtrate. The rest of the supernatant was decanted.

A further 300 ml of extracting solution was added to the bottle and the extraction was repeated. A series of rinses were carried out using deionised water.

The cleaning cycle for charcoal G60 is presented in Table 3.19. The cleaning cycle for polyclar SB100 is shown in Table 3.20.

Table 3.19. : The cleaning cycle for charcoal G60.

Cleaning cycle for charcoal G60 by the deionised water	**Cleaning cycle for charcoal G60 by 0.1 M KCl solution**	**Cleaning cycle for charcoal G60 by 0.1 M HCl solution**
Wash 1 : 300 ml deionised water. Rinse 1 : 300 ml deionised water. Rinse 2 : 300 ml deionised water . Rinse 3 : 300 ml deionised water. Rinse 4 : 300 ml deionised water.	Wash 1 : 300 ml 0.1 M KCl. Rinse 1 : 300 ml deionised water. Rinse 2 : 300 ml deionised water. Rinse 3 : 300 ml deionised water. Rinse 4 : 300 ml deionised water.	Wash 1 : 300 ml 0.1 M HCl. Rinse 1 : 300 ml deionised water. Rinse 2 : 300 ml deionised water. Rinse 3 : 300 ml deionised water . Rinse 4 : 300 ml deionised water.

Table 3.20. : The cleaning cycle for polyclar SB100.

Cleaning polyclar SB100 by the deionised water	**Cleaning polyclar SB100 by 0.1 M KCl solution**	**Cleaning polyclar SB100 by 0.1 M HCl solution**
Wash 1 : 300 ml deionised water. Wash 2 : 300 ml deionised water. Wash 3 : 300 ml deionised water. Rinse 1 : 300 ml deionised water. Rinse 2 : 300 ml deionised water. Rinse 3 : 300 ml deionised water. Rinse 4 : 300 ml deionised water. Rinse 5 : 300 ml deionised water.	Wash 1 : 300 ml 0.1 M KCl. Wash 2 : 300 ml 0.1 M KCl. Wash 3 : 300 ml 0.1 M KCl. Rinse 1 : 300 ml deionised water. Rinse 2 : 300 ml deionised water. Rinse 3 : 300 ml deionised water. Rinse 4 : 300 ml deionised water. Rinse 5 : 300 ml deionised water.	Wash 1 : 300 ml 0.1 M HCl. Wash 2 : 300 ml 0.1 M HCl. Wash 3 : 300 ml 0.1 M HCl. Rinse 1 : 300 ml deionised water. Rinse 2 : 300 ml deionised water. Rinse 3 : 300 ml deionised water. Rinse 4 : 300 ml deionised water. Rinse 5 : 300 ml deionised water.

CHEMICAL ANALYSIS :

Ammonium - N was determined by the Technicon Autoanalyzer II system as described in section 3.1.3.2. using a standard series 0.0 to 5.0 mg /l NH_4-N in deionised water.

Nitrate - N and nitrite - N were determined by the Technicon Autoanalyzer II system as described in section 2.11.1.3. Nitrite - N was determined using a standard series 0.0 to 1.0 mg /l NO_2-N in deionised water. Nitrate - N was determined using a standard series 0.0 to 5.0 mg /l NO_3-N in deionised water except the in the case of cleaning the polyclar SB100 by 0.1 M KCl solution, nitrate was determined using a standard series 0.0 to 5.0 mg /l NO_3-N in 0.1 M KCl solution since there is interference between the chloride and nitrate.

Following the extraction with deionised water, 0.1 M KCl solution and 0.1 M HCl solution, the disappearance of chloride in the subsequent water rinses was assessed by adding 4 drops of 0.05 M $AgNO_3$ to 3 ml of the filtrate.

DETERMINATION OF ANIONS IN POLYCLAR SB100 BY ION CHROMATOGRAPHY :

PROCEDURE :

5.0 g polyclar SB100 was weighed into a 60 ml glass bottle to which 50 ml deionised water was added. The bottle was shaken for 30 minutes in an orbital incubator at 25 °C, allowed to stand for 15 minutes. A portion of the supernatant was taken as sample 1, filtered through membrane filter paper (0.2 µm 47 mm membrane filter paper) using the membrane filter holder. Fluoride, chloride, nitrite, bromide, nitrate, sulphate and phosphate were determined in the filtrate using 1.0 mg /l seven anions standard solution by the DIONEX Ion Chromatography as described in section 2.15. The rest of the supernatant was decanted. This cleaning process was repeated 4 times more.

DRYING OF THE CLEANED CHARCOAL G60 OR CLEANED POLYCLAR SB100 :

The final cleaned charcoal G60 or polyclar SB100 were transferred to a 1 litre beaker with deionised water, allowed to stand for 30 minutes. The supernatant was decanted and the sample was placed in the oven at 110 °C for 18 hours in the case of the cleaned charcoal G60 and in the oven at 70 °C for approximately 2 days in the case of the cleaned polyclar SB100.

3.3.2.1.4. ADSORPTION OF NH_4-N AND NO_3-N FROM SOLUTION BY THE CLEANED CHARCOAL G60 OR POLYCLAR SB100

Two solutions, a mixed standard solution (1.0 mg /l NH_4-N + 1.0 mg /l NO_3-N) and deionised water, were tested. The mixed standard solution was prepared from $(NH_4)_2SO_4$ and $NaNO_3$.

The experimental design included 4 treatments as follows :

(a)- 2.5 g cleaned and dried absorbent + 25 ml of the mixed standard solution.

(b)- No cleaned and dried absorbent + 25 ml of the mixed standard solution .

(c)- 2.5 g cleaned and dried absorbent + 25 ml of the deionised water.

(d)- No cleaned and dried absorbent + 25 ml of the deionised water.

PROCEDURE :

The cleaned and dried absorbent plus the test solution or the test solution control was measured into a 60 ml glass bottle. The bottle was shaken for 30 minutes in an orbital incubator at 25 °C, allowed to stand for 15 minutes. The supernatant was filtered through Whatman No. 1 filter paper (pre washed as described in section 2.2.). NH_4-N and NO_3-N were determined in the filtrate. Five replicates were prepared.

In the case of the adsorption by the cleaned polyclar SB100, another experiment was carried out using mixed standard solution (10 mg /l NH_4-N + 10 mg /l NO_3-N). This experiment was carried out the same way as described above.

CHEMICAL ANALYSIS :

Ammonium - N was determined by the Technicon Autoanalyzer II system as described in section 3.1.3.2. using the mixed standard solution (1.0 mg /l NH_4-N + 1.0 mg /l NO_3-N) as a 1.0 mg /l NH_4-N standard or the mixed standard solution (10 mg /l NH_4-N + 10 mg /l NO_3-N) as a 10 mg /l NH_4-N standard and the deionised water as a 0.0 mg /l NH_4-N standard.

Nitrate was determined by the Technicon Autoanalyzer II system as described in section 2.11.1.3. using the mixed standard solution (1.0 mg /l NH_4-N + 1.0 mg /l NO_3-N) as a 1.0 mg /l NO_3-N standard or the mixed standard solution (10 mg /l NH_4-N + 10 mg /l NO_3-N) as a 10 mg /l NO_3-N standard and the deionised water as a 0.0 mg /l NO_3-N standard.

3.3.2.1.5. PREVENTION OF NO_3-N AND NH_4-N ADSORPTION BY THE CLEANED POLYCLAR SB100 USING K_2SO_4 SOLUTION

Potassium sulphate was used in the current experiment to prevent the NO_3-N and NH_4-N adsorption by the cleaned polyclar SB100.

Two solutions, a mixed standard solution (10 mg /l NH_4-N + 10 mg /l NO_3-N) in 0.25 M K_2SO_4 solution and 0.25 M K_2SO_4 solution, were tested. A mixed standard solution (20 mg /l NH_4-N + 20 mg /l NO_3-N) was prepared from $(NH_4)_2SO_4$ and $NaNO_3$ and 0.5 M K_2SO_4 solution was prepared as described in section 2.17.1.

The experimental design included 4 treatments as follows :

(a)- 2.0 g cleaned and dried polyclar SB100 + 10 ml of the mixed standard solution + 10 ml of 0.5 M K_2SO_4 solution.

(b)- No cleaned and dried polyclar SB100 + 10 ml of the mixed standard solution + 10 ml of 0.5 M K_2SO_4 solution.

(c)- 2.0 g cleaned and dried polyclar SB100 + 10 ml of the deionised water + 10 ml of 0.5 M K_2SO_4 solution.

(d)- No cleaned and dried polyclar SB100 + 10 ml of the deionised water + 10 ml of 0.5 M K_2SO_4 solution.

PROCEDURE :

The cleaned and dried polyclar SB100 plus the test solution or the test solution control was measured into a 60 ml glass bottle. The bottle was shaken for 30 minutes in an orbital incubator at 25 °C, allowed to stand for 15 minutes. The supernatant was filtered through Whatman No. 1 filter paper (pre washed). NH_4-N and NO_3-N were determined in the filtrate. Five replicates were prepared.

CHEMICAL ANALYSIS :

Ammonium - N was determined by the Technicon Autoanalyzer II system as described in section 3.1.3.2. using the mixed standard solution (10 mg /l NH_4-N + 10 mg /l NO_3-N) in 0.25 M K_2SO_4 solution as a 10 mg /l NH_4-N standard and 0.25 M K_2SO_4 solution as a 0.0 mg /l NH_4-N standard.

Nitrate was determined by the Technicon Autoanalyzer II system as described in section 2.11.1.3. using the mixed standard solution (10 mg /l NH_4-N + 10 mg /l NO_3-N) in 0.25 M K_2SO_4 solution as a 10 mg /l NO_3-N standard and 0.25 M K_2SO_4 solution as a 0.0 mg /l NH_4-N standard.

3.3.2.2. INCLUSION OF A DIALYSIS SYSTEM WITH THE TECHNICON AUTOANALYZER II FOR THE ANALYSIS OF GROUNDWATERS

This study is concerned with the inclusion of the liquid phase dialysis for the determination of nitrate-N, nitrite-N and chloride in the groundwater samples and the inclusion of the gas phase dialysis for the determination of ammonium-N in the groundwater samples.

3.3.2.2.1. INCLUSION OF THE LIQUID PHASE DIALYSIS FOR THE DETERMINATION OF NITRATE AND NITRITE IN THE GROUNDWATER SAMPLES

3.3.2.2.1.1. METHOD DEVELOPMENT

The proposed method (manifold) is shown in Figure 3.18. The samples can be run at the rate of 30 samples per hour. The colour is developed in the manifold water bath at 37 °C. The colour intensity is measured at 530 nm. The flow rates of the proposed method is shown in Table 3.21.

Table 3.21. : Reagent flow rate of the proposed method for the nitrate and nitrite nitrogen determination.

Tube	Flow rate (ml /min.)
Sample	0.80
Reducing reagent	0.42
Catalyst solution	0.42
Buffer borate	0.32
Greiss reagent	0.32
Air	0.32
Pull Through	1.20

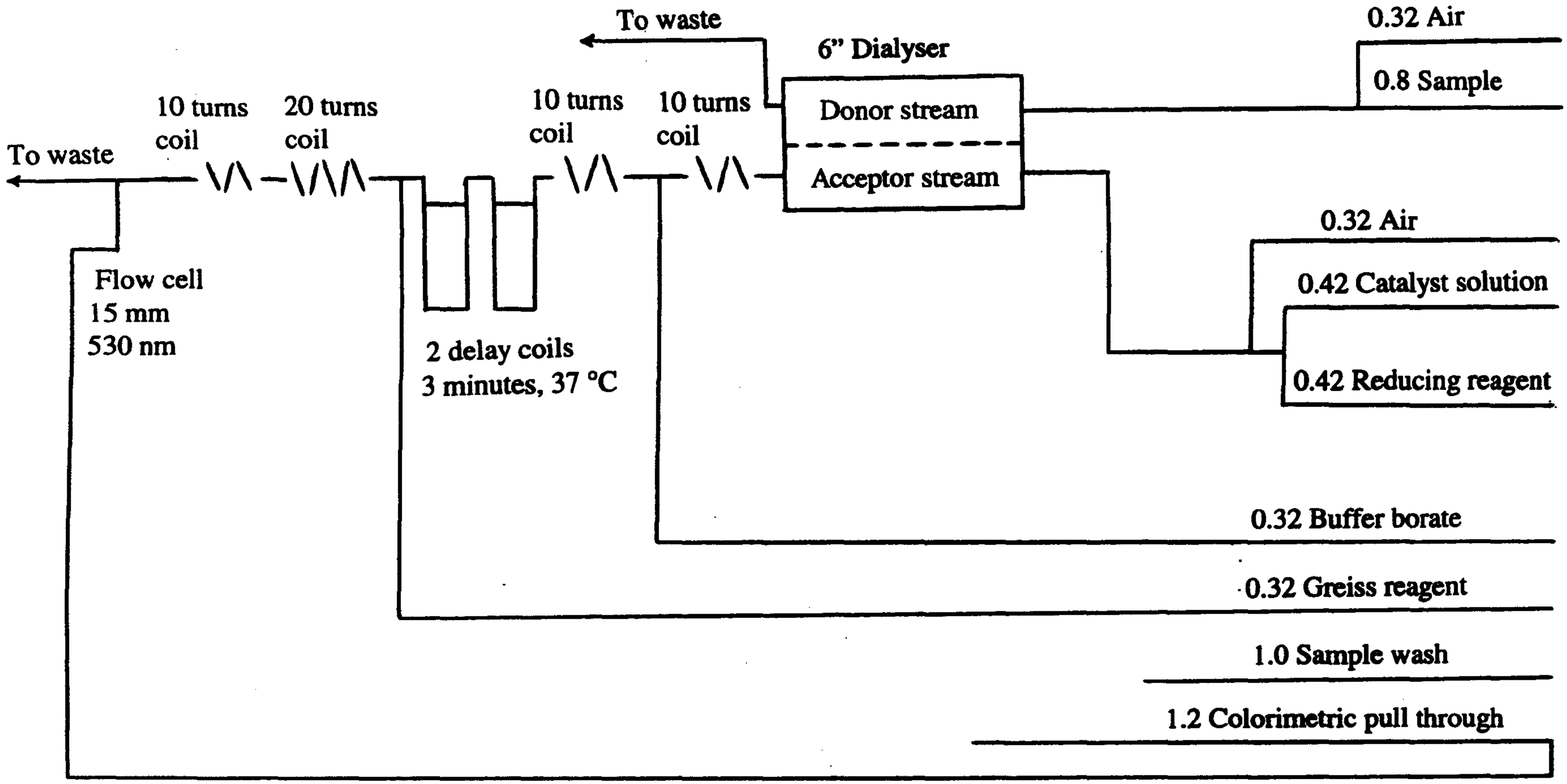


Figure 3.18. : Technicon Autoanalyzer II manifold and the dialysis system of the proposed method for the determination of NO_3-N and NO_2-N.

MATERIALS :

Dialysis block :

This was a 6 inch dialyser of the Technicon corporation, New York, USA. The part No. of the upper half and the lower half of the dialyser are 177-B077-01 and 177-B008-01, respectively.

Liquid dialysis membrane :

Technicon™ pre - Mount Dialysis Membrane Type "C" For 3" and 6" Dialysis plates. This was a product (product No. 170-0406-02) of the Technicon Industrial Corporation / Tarrytown, New York 10591, USA.

As part of the method developing, the following parameters were tested :

1- Inclusion of a wetting agent on the sample side of the dialyser.

2- Choice of the wetting agent concentration.

3- Effect of the background colour of the water sample on the determination of nitrate.

4- Evaluation of the method.

The development work was primarily concerned with obtaining satisfactory low noise levels and good peak shape.

1- INCLUSION OF A WETTING AGENT ON THE SAMPLE SIDE OF THE DIALYSER

Three modifications were carried out on the manifold. These modifications are as follows :

(a)- No source of wetting agent on the sample side of the dialyser :

The sample is connected directly to the sample side of the dialyser.

(b)- Inclusion of a wetting agent on the sample side of the dialyser :

The sample is followed by the wetting agent tube (1 ml 15 % Brij.-35 /l solution) and then connected to the sample side of the dialyser.

(c)- Inclusion of a wetting agent plus a 10 turns coil on the sample side of the dialyser :

The sample is followed by the wetting agent tube (1 ml 15 % Brij.-35 /l solution) then followed by a 10 turns coil and then connected to the upper side of the dialyser.

PROCEDURE :

This test was carried out in two procedures as follows :

Procedure (a) :

The start - up procedure was followed and the system was allowed to equilibrate. When the chart tracing reached a stable base line, 5.0 mg /l and 10 mg /l NO_3-N standard solutions were run separately with STD CAL set at 1.0 and then the peak was expanded to read 90 on the chart and each standard solution was run continuously to observe the noise level on the peak.

Procedure (b) :

The start - up procedure was followed and the system was allowed to equilibrate. When the chart tracing reached a stable base line, 10 mg /l NO_3-N standard solution was run with STD CAL set at 1.0. Then the peak was expanded to read 90 on the chart. When the chart tracing reached a stable base line again, 5 groundwater samples (Table 3.22.) were analysed using the Autoanalyzer sampler at 30 samples per hour to observe the peak shape and to ensure that a peak with a flat top was obtained.

MATERIALS :

The groundwater samples which have been analysed in the current test are from Nobel Enterprises and are shown in Table 3.22.

Table 3.22. : Description of the groundwater samples.

Well No.	Sampling date
New nylon site well	24 / 9 / 1996
TW 2	2 / 5 / 1996
SW 11	20 / 3 / 1996
SW 15	24 / 4 / 1996
SW 16	11 / 4 / 1996

2- CHOICE OF THE WETTING AGENT CONCENTRATION

Three wetting agent solutions, 1 ml, 2 ml and 5 ml 15 % Brij.-35 /l solutions were tested to select the best wetting agent concentration which gives a smooth flow.

PROCEDURE :

The start - up procedure was followed and the system was allowed to equilibrate with one of the wetting agent concentration in use. When the chart tracing reached a stable base line, 10 mg /l NO_3-N standard solution was run with STD CAL set at 1.0 with one of the wetting agent concentration in use, then the peak was expanded to read 90 on the chart and the 10 mg /l NO_3-N standard solution was run continuously to observe the noise level on the peak. This was repeated for 1 ml, 2 ml and 5 ml 15 % Brij.-35 /l solutions.

3- EFFECT OF THE BACKGROUND COLOUR OF THE WATER SAMPLE ON THE DETERMINATION OF NITRATE

This test was carried out to determine the background colour of the water samples.

PROCEDURE :

The start - up procedure was followed and the system was allowed to equilibrate. When the chart tracing reached a stable base line, 10 mg /l NO_3-N standard

solution was run with STD CAL set at 1.0. Then the peak was expanded to read 90 on the chart. When the chart tracing reached a stable base line, the Greiss reagent was replaced with 10 % HCl solution and the base line was reset again. When the chart tracing reached a stable base line, the 5 groundwater samples (Table 3.22.) were analysed as diluted and undiluted samples using the Autoanalyzer sampler at 30 samples per hour.

4- EVALUATION OF THE METHOD

Three modifications were carried out on the manifold. These modifications are as follows :

(a)- Addition of the catalyst solution and reducing reagent solution on the acceptor side of the dialyser :

The catalyst solution and reducing reagent solution were connected to the acceptor side of the dialyser and the buffer borate solution was added after the dialyser.

(b)- Addition of the buffer borate and reducing reagent solutions on the acceptor side of the dialyser :

The buffer borate and reducing reagent solutions were connected to the acceptor side of the dialyser and the catalyst solution was added after the dialyser.

(c)- Addition of the buffer borate solution on the acceptor side of the dialyser :

The buffer borate solution was connected to the acceptor side of the dialyser and the catalyst solution and reducing reagent solution were added after the dialyser.

PROCEDURE :

The start - up procedure was followed and the system was allowed to equilibrate. When the chart tracing reached a stable base line, 10 mg /l NO_3-N standard solution was run with STD CAL set at 1.0. Then the peak was expanded to read 90 on the chart. When the chart tracing reached a stable base line again, 2, 4, 6, 8 and 10 mg /l NO_3-N standard solutions were analysed in duplicate using the Autoanalyzer sampler at 30 samples per hour.

3.3.2.2.2. INCLUSION OF THE LIQUID PHASE DIALYSIS FOR THE DETERMINATION OF CHLORIDE IN THE GROUNDWATER SAMPLES

The final method for the determination of chloride in the groundwater samples is described in section 3.3.3.2.4. This method was evaluated as follows :

1- EVALUATION OF THE METHOD

PROCEDURE :

The start - up procedure was followed and the system was allowed to equilibrate. When the chart tracing reached a stable base line, 100 mg /l Cl standard solution was run with STD CAL set at 1.0. Then the peak was expanded to read 90 on the chart. When the chart tracing reached a stable base line again, 20, 40, 60, 80 and 100 mg /l Cl standard solutions were analysed in duplicate using the Autoanalyzer sampler at 50 samples per hour.

3.3.2.2.3. INCLUSION OF THE GAS PHASE DIALYSIS FOR THE DETERMINATION OF AMMONIUM IN THE GROUNDWATER SAMPLES

Ammonium was determined in the groundwater samples by including gas phase dialysis with Technicon Autoanalyzer II system as recommended by Khan (1994). The dialysis system is shown in Figure 3.19.

REAGENTS:

As described in section 3.1.3.2. and the additional reagents were as follows :

1 M Sodium hydroxide :

40.0 g sodium hydroxide was dissolved in approximately 500 ml of deionised water in a 1 litre volumetric flask. After cooling the volume was made up to the mark with deionised water.

2 M Hydrochloric acid :

173 ml concentrated hydrochloric acid was diluted with deionised water in 1 litre volumetric flask and the volume was made up to the mark with deionised water.

0.01 M Hydrochloric acid :

10 ml of 1 M hydrochloric acid were pipetted into a 1 litre volumetric flask and the volume was made up to the mark with deionised water.

The solutions were analysed for ammonium using the manifold shown in Figure 3.19. along with standard solutions, blanks and zeros. The samples were run at the rate of 20 per hour. The colour was developed in the manifold water bath at 37 °C. The colour intensity was measured at 650 nm. The air was cleaned of atmospheric

ammonia by bubbling through 5 % HCl solution. Ammonium has a linear calibration graph in the range 0.0 to 10 mg /l NH_4-N.

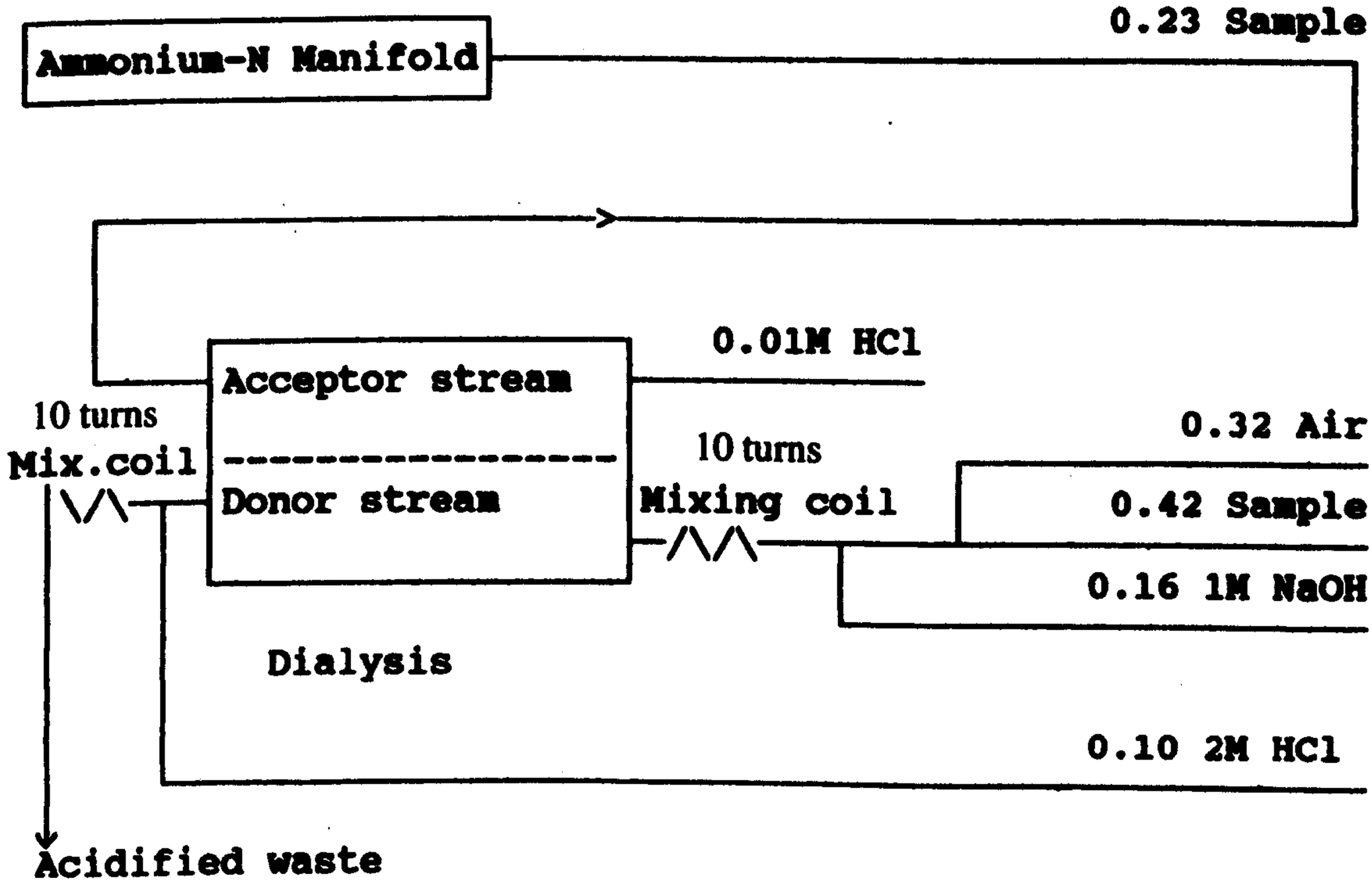


Figure 3.19. : Technicon Autoanalyzer II manifold and the dialysis system for the determination of NH_4-N.

3.3.3. RESULTS AND DISCUSSION

3.3.3.1. USING CHARCOAL G60 AND POLYCLAR SB100 FOR THE DECOLORISATION OF THE WATER SAMPLES

The efficiency of charcoal G60 and polyclar SB100 in the decolorisation of the water samples is presented in Table 3.23. The results obtained showed that the 0.5 g of either charcoal or polyclar was ineffective. Although the 2.5 g charcoal was effective in the decolorisation, particularly with well water No. SW 15, 2.5 g polyclar or even with higher level (5.0 g) was ineffective particularly with well water No. SW 16.

Table 3.23. : Efficiency of charcoal G60 and polyclar SB100 in the decolorisation of the water samples.

Decolorisation treatment	Well water No. SW 15	Well water No. SW 16
0.5 g charcoal G60 + 25 ml water sample	Coloured	Coloured
2.5 g charcoal G60 + 25 ml water sample	Clear	Slightly coloured
0.5 g polyclar SB100 + 25 ml water sample	Coloured	Coloured
2.5 g polyclar SB100 + 25 ml water sample	Slightly coloured	Coloured
5.0 g polyclar SB100 + 25 ml water sample	----------	Coloured

Similar results were obtained by some authors such as McGeehan et al. (1989) who found that the errors in the colorimetric analysis decreased with increasing the addition of decolorising charcoal.

Simpson et al. (1982) found that the removal of the natural colour of wine was more by decolorising carbon than by polyvinylpyrrolidone (PVPP).

Table 3.24. illustrates the levels of impurities in the charcoal G60 and polyclar SB100. All results are the mean of 5 replicates. As can be seen, charcoal had higher levels of metals such as potassium, sodium and calcium than polyclar. The main nitrogen contaminants are ammonium-N and nitrate-N in the charcoal and nitrate-N and nitrite-N in the polyclar.

Table 3.24. : Levels of impurities in charcoal G60 and polyclar SB100.

Component (mg /kg)	Charcoal G60	Polyclar SB100
Ammonium-N	0.5	0.6
Nitrate-N	7.3	19.2
Nitrite-N	0.5	7.7
Potassium	14.0	11.8
Sodium	675	209
Calcium	42.1	37.4
Magnesium	3.2	3.1

Because of the high levels of contaminants, it was decided to clean charcoal G60 from the ammonium-N and nitrate-N and polyclar SB100 from the nitrate-N and nitrite-N.

The cleaning of charcoal G60 by deionised water is shown in Table 3.25., by 0.1 M KCl solution in Table 3.26. and by 0.1 M HCl solution in Table 3.27. As can be seen, in all cases the cleaning was similar. The contaminants fell rapidly to low levels of NH_4-N and NO_3-N. The levels of contaminants were higher in the initial wash and the first 2 rinses and then fell to low levels. The contaminants were removed in the first 2 rinses but small amounts were found in the later rinses and these were removed in the final rinse particularly NO_3-N.

Table 3.25. : Cleaning charcoal G60 from impurities (NH_4-N and NO_3-N) by the deionised water.

Treatment	NH_4-N (mg /l)	NO_3-N (mg /l)
300 ml water [wash 1]*	9.20	3.30
300 ml water [rinse 1]	1.79	1.17
300 ml water [rinse 2]	0.47	0.48
300 ml water [rinse 3]	0.25	0.41
300 ml water [rinse 4]	0.11	0.27

* Time period of shaking in the orbital incubator at 25 °C is 30 minutes for wash 1 and for rinse 1, 2, 3 and 4.

Table 3.26. : Cleaning charcoal G60 from impurities (NH_4-N and NO_3-N) by 0.1 M KCl solution.

Treatment	NH_4-N (mg /l)	NO_3-N (mg /l)
300 ml 0.1 M KCl [wash 1]*	10.80	6.90
300 ml deionised water [rinse 1]	2.52	1.87
300 ml deionised water [rinse 2]	0.51	0.39
300 ml deionised water [rinse 3]	0.16	0.31
300 ml deionised water [rinse 4]	0.08	0.22

* Time period of shaking in the orbital incubator at 25 °C is 30 minutes for wash 1 and for rinse 1, 2, 3 and 4.

Table 3.27. : Cleaning charcoal G60 from impurities (NH_4-N and NO_3-N) by 0.1 M HCl solution.

Treatment	NH_4-N (mg /l)	NO_3-N (mg /l)
300 ml 0.1 M HCl [wash 1]**	ND*	ND
300 ml deionised water [rinse 1]	2.92	0.77
300 ml deionised water [rinse 2]	0.66	0.40
300 ml deionised water [rinse 3]	0.16	0.26
300 ml deionised water [rinse 4]	0.07	0.21

* ND = Not determined.

** Time period of shaking in the orbital incubator at 25 °C is 30 minutes for wash 1 and for rinse 1, 2, 3 and 4.

Table 3.28. displays the level of chloride in the water rinses used in the cleaning of charcoal G60. As can be seen, chloride is not completely removed even after 4 rinses.

Table 3.28. : Level of chloride in the water rinses used in the cleaning of charcoal G60.

Treatment	Degree of precipitation
2- Cleaning charcoal G60 by 0.1 M KCl :	
300 ml deionised water [rinse 1]	+ + +
300 ml deionised water [rinse 2]	+ + +
300 ml deionised water [rinse 3]	+ +
300 ml deionised water [rinse 4]	+
3- Cleaning charcoal G60 by 0.1 M HCl :	
300 ml deionised water [rinse 1]	+ + +
300 ml deionised water [rinse 2]	+ + +
300 ml deionised water [rinse 3]	+ +
300 ml deionised water [rinse 4]	+

The cleaning of polyclar SB100 by deionised water is shown in Table 3.29., by 0.1 M KCl solution in Table 3.30. and by 0.1 M HCl solution in Table 3.31. It can be seen from the results obtained that the levels of contaminants are lower in the initial washes compared to that of charcoal G60. The contaminants fell rapidly to low levels of NO_3-N and NO_2-N.

Table 3.29. : Cleaning polyclar SB100 from impurities (NO_3-N and NO_2-N) by the deionised water.

Treatment	NO_3-N (mg /l)	NO_2-N (mg /l)
300 ml deionised water [wash 1]*	1.22	0.65
300 ml deionised water [wash 2]	0.14	0.09
300 ml deionised water [wash 3]	0.05	0.02
300 ml deionised water [rinse 1]	0.03	0.01
300 ml deionised water [rinse 2]	0.01	0.00
300 ml deionised water [rinse 3]	0.04	0.01
300 ml deionised water [rinse 4]	0.03	0.01
300 ml deionised water [rinse 5]	0.02	0.01

* Time period of shaking in the orbital incubator at 25 °C is 30 minutes for wash 1, 2 and 3 and for rinse 1, 2, 3, 4 and 5.

Table 3.30. : Cleaning polyclar SB100 from impurities (NO_3-N and NO_2-N) by 0.1 M KCl solution.

Treatment	NO_3-N (mg /l)	NO_2-N (mg /l)
300 ml 0.1 M KCl [wash 1]*	1.38	0.56
300 ml 0.1 M KCl [wash 2]	0.20	0.09
300 ml 0.1 M KCl [wash 3]	0.12	0.07
300 ml deionised water [rinse 1]	-0.01	0.01
300 ml deionised water [rinse 2]	0.06	0.00
300 ml deionised water [rinse 3]	0.01	0.02
300 ml deionised water [rinse 4]	-0.01	0.01
300 ml deionised water [rinse 5]	-0.01	0.01

* Time period of shaking in the orbital incubator at 25 °C is 30 minutes for wash 1, 2 and 3 and for rinse 1, 2, 3, 4 and 5.

Table 3.31. : Cleaning polyclar SB100 from impurities (NO_3-N and NO_2-N) by 0.1 M HCl solution.

Treatment	NO_3-N (mg /l)	NO_2-N (mg /l)
300 ml 0.1 M HCl [wash 1]**	ND*	ND
300 ml 0.1 M HCl [wash 2]	ND	ND
300 ml 0.1 M HCl [wash 3]	ND	ND
300 ml deionised water [rinse 1]	0.07	0.00
300 ml deionised water [rinse 2]	0.03	0.00
300 ml deionised water [rinse 3]	0.03	-0.00
300 ml deionised water [rinse 4]	0.05	0.00
300 ml deionised water [rinse 5]	0.05	0.00

* ND = Not determined.

** Time period of shaking in the orbital incubator at 25 °C is 30 minutes for wash 1, 2 and 3 and for rinse 1, 2, 3, 4 and 5.

Table 3.32. shows the level of chloride in the water rinses used in the cleaning of polyclar SB100. As can be seen, there is no chloride in the polyclar SB100. Chloride was not effectively removed following washing with 0.1 M KCl or 0.1 M HCl solution.

Cleaning the polyclar SB100 produced better final levels of NO_3-N and NO_2-N than cleaning of charcoal G60 but the cleaning should be carried out with deionised water only to avoid chloride contamination.

Table 3.32. : Level of chloride in the water rinses used in the cleaning of polyclar SB100.

Treatment	Degree of precipitation
1- Cleaning polyclar SB100 by deionised water : 300 ml deionised water [wash 1]	0
2- Cleaning polyclar SB100 by 0.1 M KCl : 300 ml deionised water [rinse 1] 300 ml deionised water [rinse 2] 300 ml deionised water [rinse 3] 300 ml deionised water [rinse 4] 300 ml deionised water [rinse 5]	+++ +++ +++ +++ +++
3- Cleaning polyclar SB100 by 0.1 M HCl : 300 ml deionised water [rinse 1] 300 ml deionised water [rinse 2] 300 ml deionised water [rinse 3] 300 ml deionised water [rinse 4] 300 ml deionised water [rinse 5]	+++ +++ +++ +++ +++

CLEANING POLYCLAR SB100 FROM THE ANIONS :

The anions eluted from polyclar SB100 are shown in Table 3.33. From the results obtained, fluoride can not be detected reliably because two small peaks which were probably propionate, lactate, acetate, butyrate or format co-eluted with a small fluoride peak. These two organic acids were frequently found as trace contaminants in glasswares and plasticwares possibly as a result of the cleaning process.

The results had also shown that there was a high level of phosphate in the polyclar SB100 and low levels of chloride and sulphate. It is possible to reduce the anions to acceptable levels using the deionised water only.

Table 3.33. : Cleaning polyclar SB100 from the anions by deionised water.

Component (mg /l)	50 ml deionised water [wash 1]	50 ml deionised water [wash 2]	50 ml deionised water [wash 3]	50 ml deionised water [wash 4]	50 ml deionised water [wash 5]
Fluoride	0.00	0.00	0.00	0.00	0.00
Chloride	0.41	0.07	0.03	0.02	0.01
Nitrite	1.13	0.19	0.08	0.03	0.02
Bromide	0.00	0.00	0.00	0.00	0.00
Nitrate	0.24	0.04	0.01	0.00	0.00
Sulphate	0.61	0.11	0.04	0.02	0.01
Phosphate	3.42	0.53	0.21	0.07	0.05

The adsorption and desorption of NH_4-N and NO_3-N by the cleaned charcoal is listed in Table 3.34. All the results are the mean of 5 replicates. The results obtained showed a large extraction of ammonium with deionised water and with the mixed standard solution which is much greater than the final rinses in the washing process. This ammonium-N contamination could be as a result of the adsorption of ammonia gas during the drying of the charcoal or the release of ammonium due to decomposition of some components in the charcoal while drying or the release of ammonium physically held on the charcoal which had not removed by the cleaning process. There is a slight adsorption of ammonium by the filter paper. There was a small amount of nitrate extracted from the charcoal with deionised water similar to that of the final rinses. There was a serious adsorption of nitrate by the cleaned charcoal from the mixed standard solution. The filter paper gave a complete recovery of nitrate.

Table 3.34. : Adsorption and desorption of NH_4-N and NO_3-N by the cleaned charcoal G60.

Treatment	NH_4-N (mg /l)	NO_3-N (mg /l)
Charcoal G60 + mixed standard solution (1.0 mg /l NH_4-N + 1.0 mg /l NO_3-N).	1.69	0.21
Control + mixed standard solution (1.0 mg /l NH_4-N + 1.0 mg /l NO_3-N).	0.88	1.02
Charcoal G60 + deionised water.	0.62	0.13

Charcoal G60 is not suitable to be used for decolorising the water samples since the cleaned charcoal became contaminated with ammonium and adsorbed nitrate from the solution.

The adsorption and desorption of NH_4-N and NO_3-N by the cleaned polyclar is given in Table 3.35. and Table 3.36. All the results are the mean of 5 replicates. From the results obtained, there was a slight extraction of ammonium from the polyclar SB100 with deionised water and there was a slight adsorption of ammonium by the filter papers. There was a slight extraction of nitrate from the polyclar with deionised water similar to that of the final rinses but polyclar adsorbed NO_3-N from the mixed standard solution.

Table 3.35. : Adsorption and desorption of NH_4-N and NO_3-N by the cleaned polyclar SB100.

Treatment	NH_4-N (mg /l)	NO_3-N (mg /l)
Polyclar SB100 + mixed standard solution (1.0 mg /l NH_4-N + 1.0 mg /l NO_3-N).	1.00	0.76
Control + mixed standard solution (1.0 mg /l NH_4-N + 1.0 mg /l NO_3-N).	0.84	1.01
Polyclar SB100 + deionised water.	0.08	0.05

Table 3.36. : Adsorption and desorption of NH_4-N and NO_3-N by the cleaned polyclar SB100.

Treatment	NH_4-N (mg /l)	NO_3-N (mg /l)
Polyclar SB100 + mixture standard solution (10 mg /l NH_4-N + 10 mg /l NO_3-N).	9.73	7.83
Control + mixture standard solution (10 mg /l NH_4-N + 10 mg /l NO_3-N).	9.77	9.97
Polyclar SB100 + deionised water.	0.10	0.04

Table 3.37. showed that K_2SO_4 solution prevented the ammonium adsorption by the filter paper and the polyclar but not the adsorption of nitrate.

Table 3.37. : Prevention of NO_3-N and NH_4-N from the adsorption by the cleaned polyclar SB100 using electrolyte (0.5 M K_2SO_4 solution).

Treatment	NH_4-N (mg /l)	NO_3-N (mg /l)
Polyclar SB100 + mixture standard solution (20 mg /l NH_4-N + 20 mg /l NO_3-N) + 0.5 M K_2SO_4 solution.	10.16	8.52
Control + mixture standard solution (20 mg /l NH_4-N + 20 mg /l NO_3-N) + 0.5 M K_2SO_4 solution.	10.00	10.01
Polyclar SB100 + deionised water + 0.5 M K_2SO_4 solution.	0.08	0.07

From this study, it was decided not to use charcoal G60 or polyclar SB100 as decolorising agents for the water samples.

3.3.3.2. INCLUSION OF A DIALYSIS SYSTEM WITH THE TECHNICON AUTOANALYZER II FOR THE ANALYSIS OF GROUNDWATERS

3.3.3.2.1. INCLUSION OF THE LIQUID PHASE DIALYSIS FOR THE DETERMINATION OF NITRATE AND NITRITE IN THE GROUNDWATER SAMPLES

1- INCLUSION OF A WETTING AGENT IN THE SAMPLE SIDE OF THE DIALYSER

Table 3.38. shows the assessment of the inclusion of a wetting agent on the sample side of the dialyser. Based on the peak shape and the noise level in the peak, it was decided to include a wetting agent plus a 10 turns coil on the sample side of the dialyser.

Table 3.38. : Assessment of the inclusion of a wetting agent on the sample side of the dialyser.

Inclusion of a wetting agent on the sample side of the dialyser	Noise level in the continuous peak	Peak shape of the individual peaks	Noise level in the individual peaks
1- No source of wetting agent	Poor	Poor (pointed)	Poor
2- Inclusion of a wetting agent	Poor	Borderline	Acceptable
3- Inclusion of a wetting agent plus a 10 turns coil	Borderline	Acceptable	Acceptable

2- CHOICE OF THE WETTING AGENT CONCENTRATION

The noise level in the continuous peak for each wetting agent concentration is illustrated in Table 3.39. The minimum noise level in the peak was obtained by 5 ml 15 % Brij.-35 /l solution, therefore it was decided to use this wetting agent concentration.

Table 3.39. : Assessment of the wetting agent concentration.

Wetting agent concentration	Noise level in the continuous peak
(a)- 1 ml 15 % Brij.-35 /l solution	Poor
(b)- 2 ml 15 % Brij.-35 /l solution	Poor
(c)- 5 ml 15 5 Brij.-35 /l solution	Borderline

3- EFFECT OF THE BACKGROUND COLOUR OF THE WATER SAMPLE ON THE DETERMINATION OF NITRATE

The background colour of the groundwater samples is given in Table 3.40. From the results obtained there was no background colour for both the undiluted and diluted groundwater samples. Therefore, there is no need to make the colour correction for the results.

Table 3.40. : The background colour of the groundwater samples.

Groundwater samples	Background colour
Undiluted samples	No colour
Diluted samples	No colour

4- EVALUATION OF THE METHOD

The linearity of the method with different additions of the reagents is presented in Figures 3.20., 3.21. and 3.22. It was observed from the results that the addition of the catalyst solution and reducing reagent solution on the acceptor side of the dialyser gave a curved calibration graph. This curved calibration graph is attributed to the low concentration of copper catalyst (3.14 mg Cu /l) in the acceptor stream passing

through the dialyser membrane from the acceptor side to the sample side of the dialyser and being lost, therefore, the colour is not developing fully. Although both the addition of the buffer borate and reducing reagent solutions on the acceptor side of the dialyser and the addition of the buffer borate solution on the acceptor side of the dialyser gave a linear calibration graph, it was decided to add the buffer borate and reducing reagent solutions on the acceptor side of the dialyser since it was more convenient.

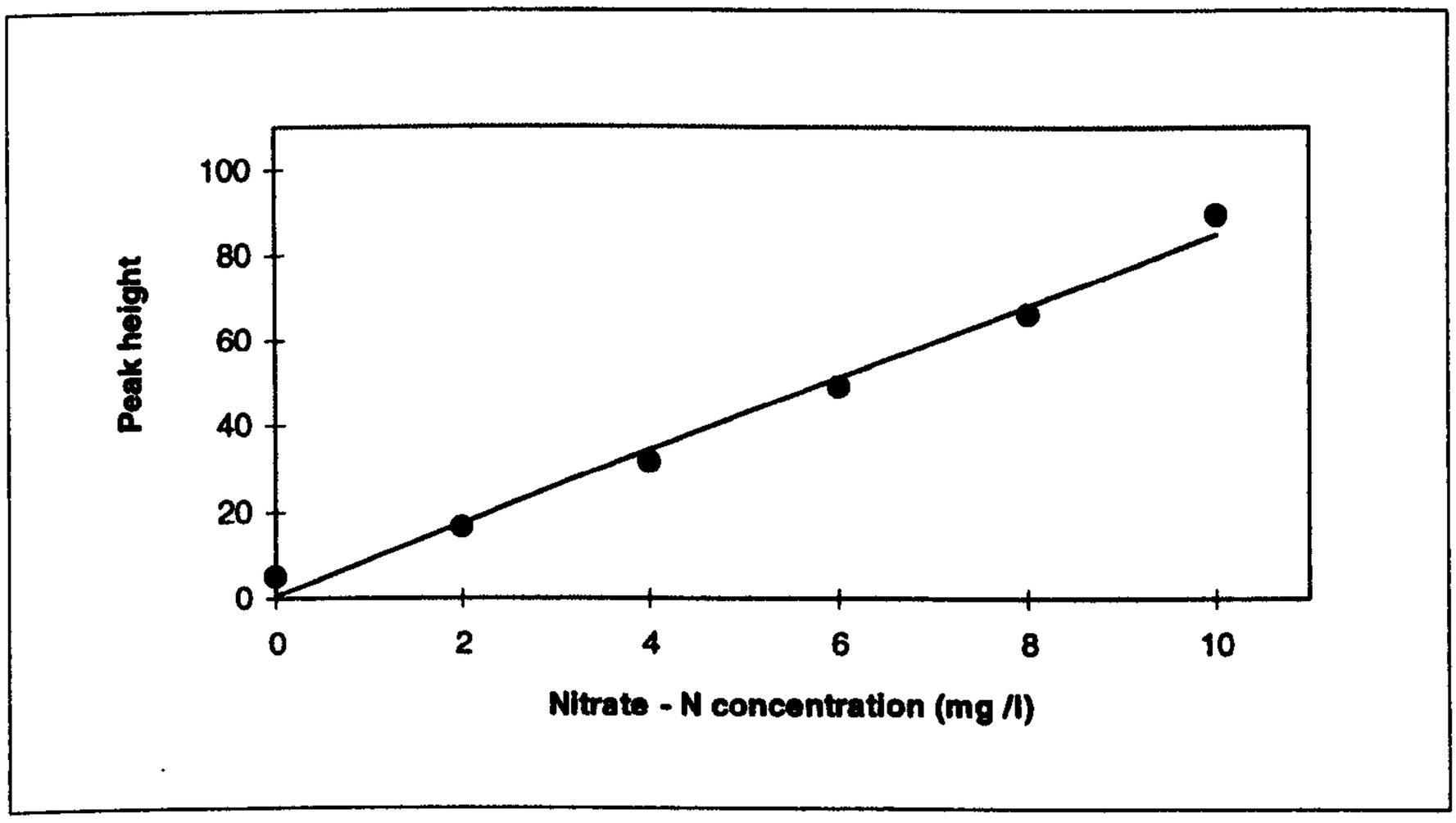


Figure 3.20. : Linearity of the method with the addition of the catalyst solution and reducing reagent solution on the acceptor side of the dialyser.

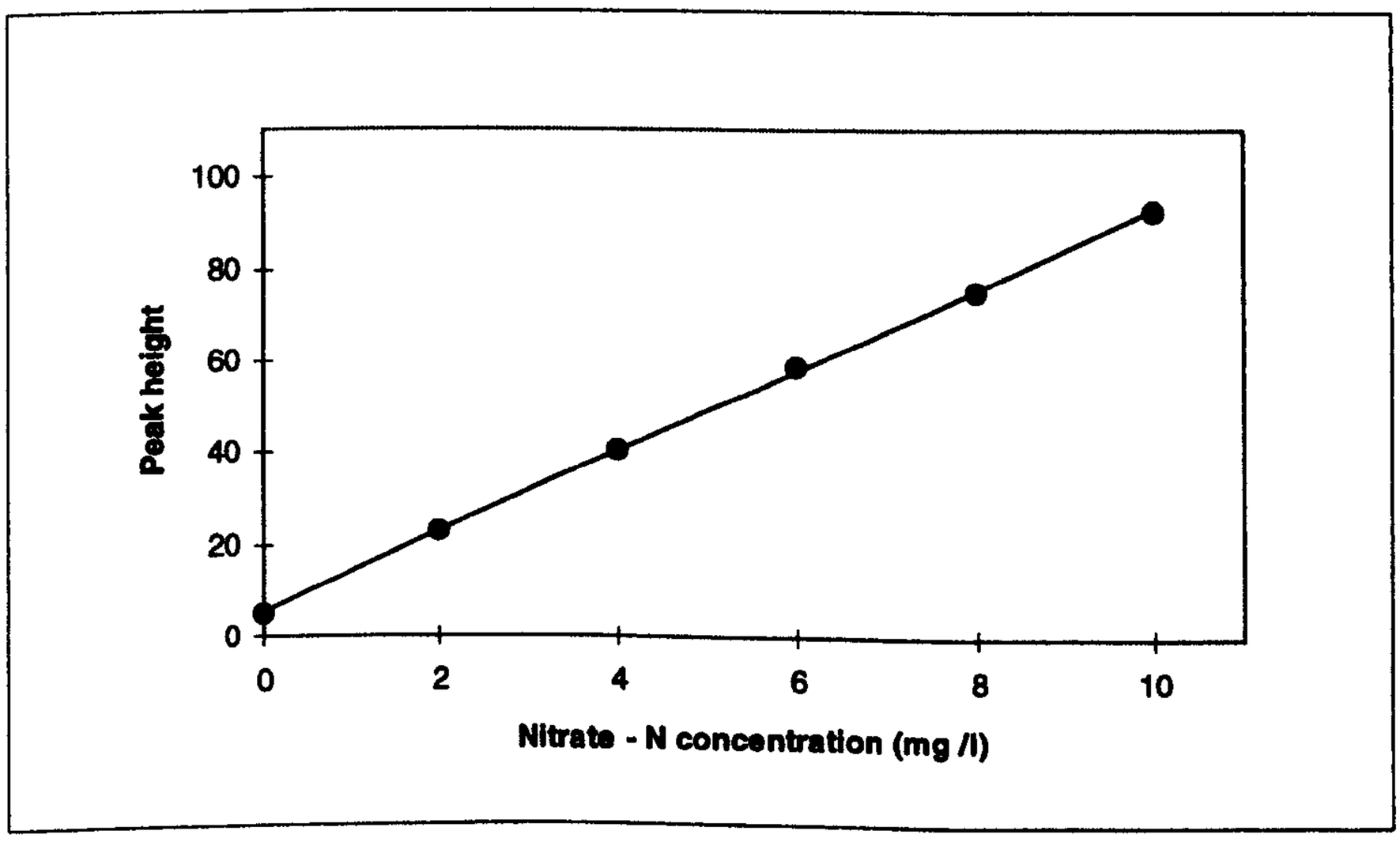


Figure 3.21. : Linearity of the method with the addition of the buffer borate and reducing reagent solutions on the acceptor side of the dialyser.

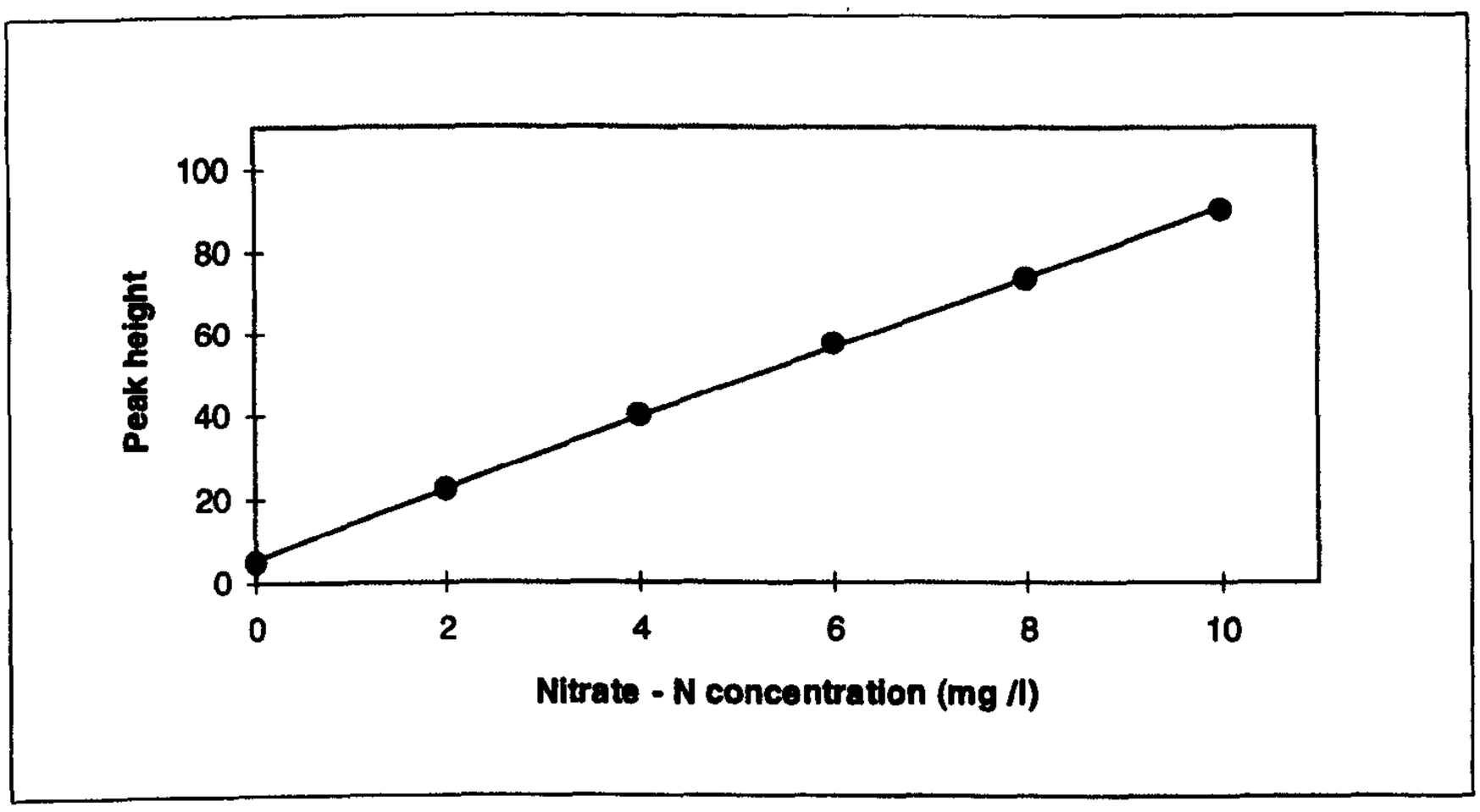


Figure 3.22. : Linearity of the method with the addition of the buffer borate solution on the acceptor side of the dialyser.

3.3.3.2.2. FINAL METHOD FOR THE DETERMINATION OF NITRATE AND NITRITE IN THE GROUNDWATER SAMPLES

REAGENTS :

As described in section 2.11.1.3.

PROCEDURE :

The manifold (Figure 3.23.) can be used for the determination of nitrate and nitrite nitrogen in water and a range of extracting solutions.

Table 3.41. : Reagent flow rate of the final method for the nitrate and nitrite nitrogen determination.

Tube	Flow rate (ml /min.)
Sample	0.80
Reducing reagent	0.42
Catalyst solution	0.42
Buffer borate	0.32
Greiss reagent	0.32
Wetting agent solution	0.10
Air	0.32
Pull Through	1.20

The solutions can be analysed for nitrate using the manifold shown in Figure 3.23. along with standard solutions, blanks and zeros. The samples can be run at the rate of 30 per hour. The colour is developed in the manifold water bath at 37 °C. The colour intensity is measured at 530 nm. Nitrate has a linear calibration graph in the range 0.0 to 10 mg /l NO_3-N. Nitrite has a linear calibration graph in the range 0.0 to 4.0 mg /l NO_2-N.

For the determination of nitrite nitrogen, the reducing reagents are replaced with nitrogen free deionised water containing 1 ml per litre of 15 % Brij.-35 solution.

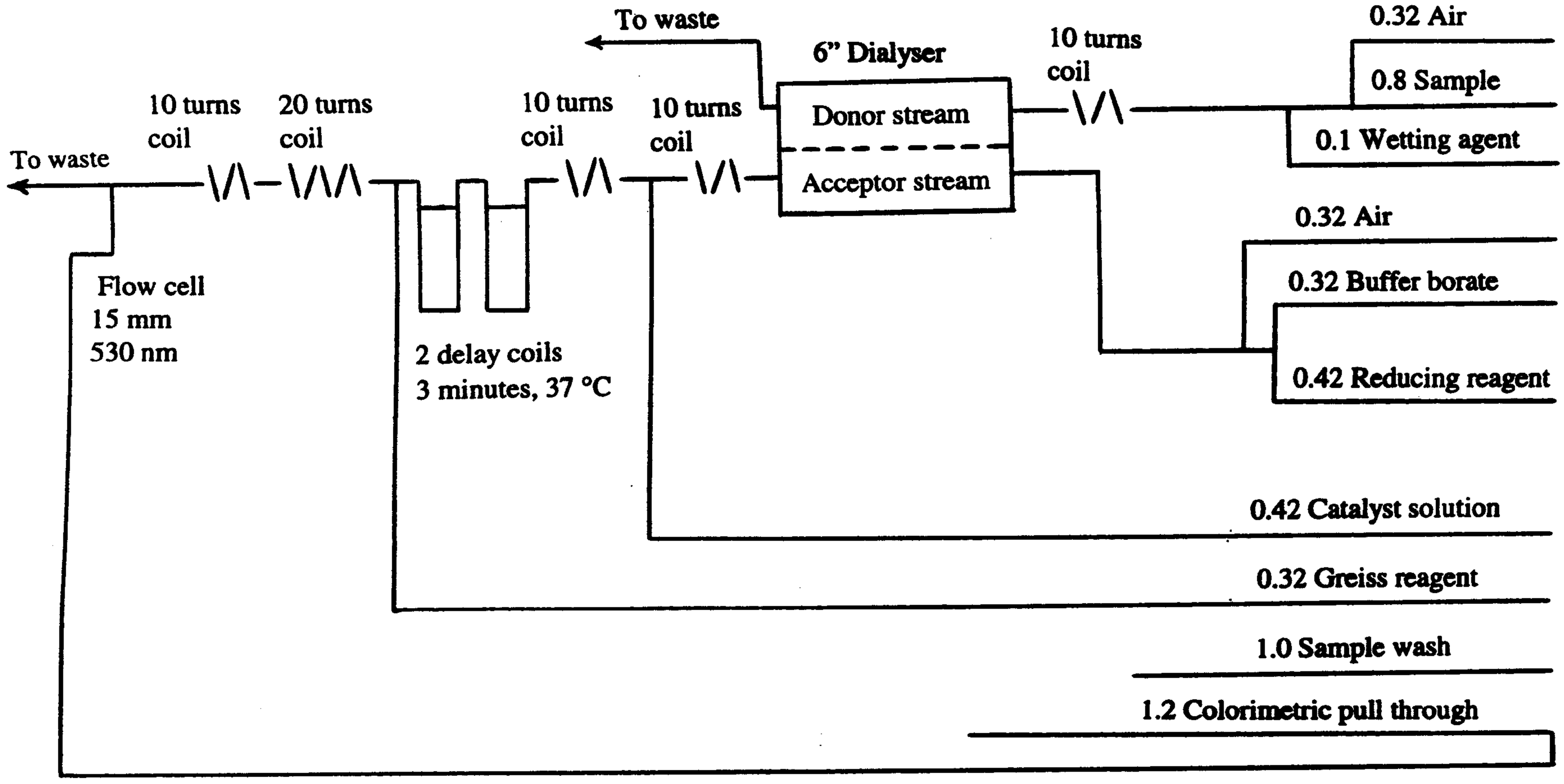


Figure 3.23. : Technicon Autoanalyzer II manifold and the dialysis system of the final method for the determination of NO_3-N and NO_2-N.

3.3.3.2.3. INCLUSION OF THE LIQUID PHASE DIALYSIS FOR THE DETERMINATION OF CHLORIDE IN THE GROUNDWATER SAMPLES

The linearity of the method used for the determination of chloride in the groundwater samples is shown in Figure 3.24. It can be seen that the method had a linear calibration graph. Very slight curvature was a characteristic of this method (A NON, 1982).

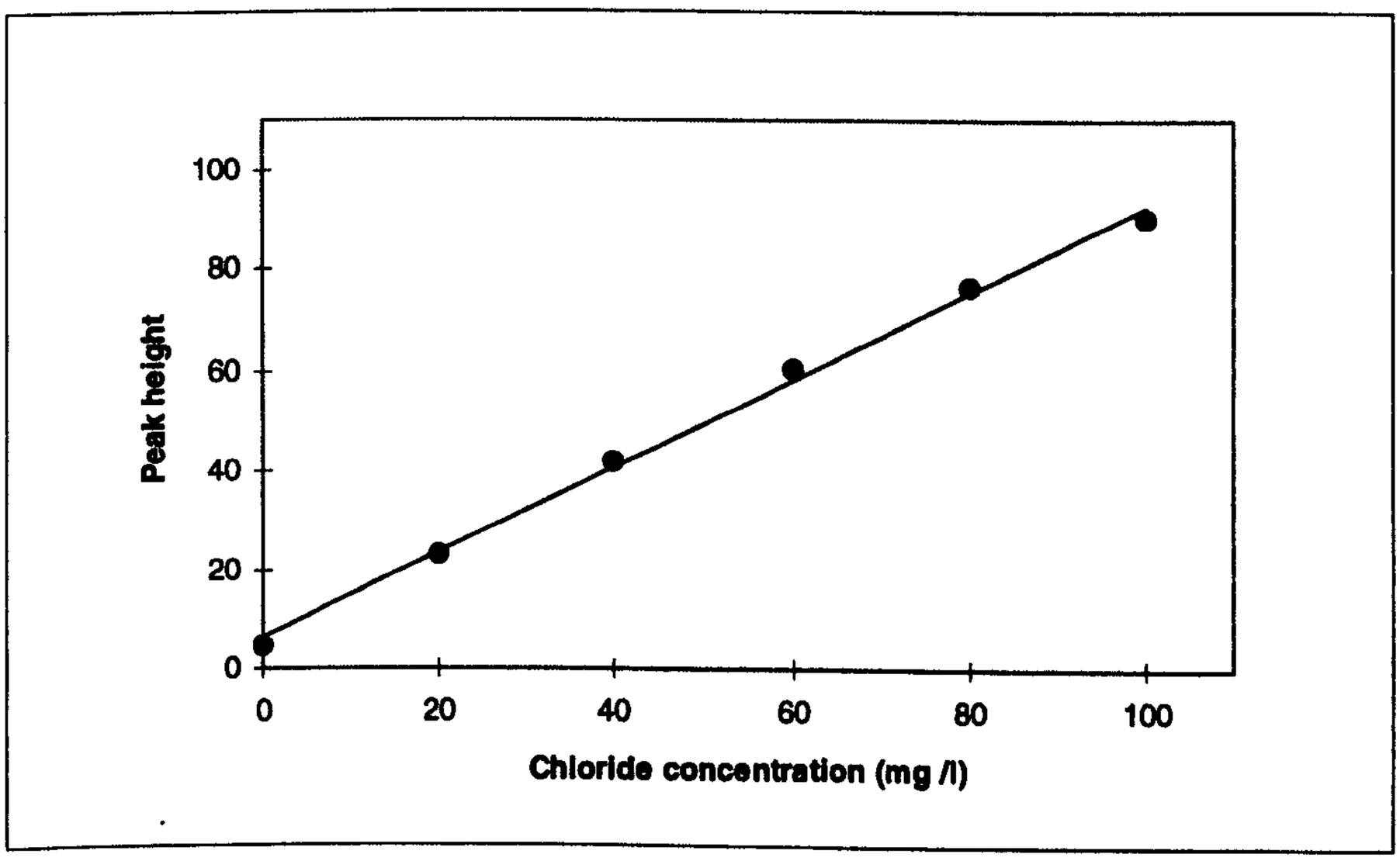


Figure 3.24.: Linearity of the method used for the determination of chloride in the groundwater samples.

3.3.3.2.4. FINAL METHOD FOR THE DETERMINATION OF CHLORIDE IN THE GROUNDWATER SAMPLES

Chloride reacts with the soluble unionised compound mercuric thiocyanate to produce the more stable soluble complex mercuric chloride whereby releasing the free thiocyanate anion into solution. The free thiocyanate reacts with ferric ions in nitric acid solution to produce a highly coloured red ferric thiocyanate complex. The method is applicable to water samples in the range 0.0 to 10 mg /l and 0.0 to 100 mg /l (A NON, 1982).

REAGENTS :

High quality deionised water and analar quality reagents were used where possible. These reagents were prepared in a fume cupboard. A fume cupboard was cleared and a 2 figure top pan balance was set up. Care was taken to ensure that the balance was properly locked before attempting to move it. The fan was switched off while actual weighing was in progress but otherwise was kept running. Any spills were cleared up immediately. When finished, the balance was wiped down and returned to the balance bench. Finally, the fume cupboard was wiped down.

(a)- Mercury thiocyanate ferric nitrate reagent :

***** Mercuric thiocyanate is highly toxic. Nitric acid is highly corrosive. This reagent should be prepared in a fume cupboard. *****

2.5 ml concentrated nitric acid was added in a 50 ml beaker to which 0.5 ml deionised water was added. The beaker was partly covered with a watch glass to minimise losses of nitric acid and boiled gently for 15 minutes on hot plate and then cooled to room temperature. 0.3 g mercuric thiocyanate was weighed into a 250 ml beaker and dissolved in 75 ml methanol and then filtered through a Whatman No. 42 filter paper directly into a 250 ml volumetric flask. 15 g ferric nitrate was weighed into a 250 ml beaker and dissolved in 100 ml deionised water. The boiled, cooled nitric acid was added to the filtered mercuric thiocyanate solution filtering through the same filter

paper. The volume was made up to the mark with deionised water. This solution was degassed for 10 minutes in the ultrasonic bath.

(b)- 1 M HNO_3 :

64.31 ml of concentrated nitric acid was diluted with deionised water in 1 litre volumetric flask. 2 ml 15 % Brij.-35 solution was added to the diluted nitric acid solution and the volume was made up to the mark with deionised water.

(c)- Wetting agent solution :

1 ml of 15 % Brij.-35 solution was added to 1 litre of deionised water in 1 litre volumetric flask. This solution was degassed for 10 minutes in the ultrasonic bath.

(d)- Chloride standard stock solution (1000 mg /l Cl) :

Sodium chloride was dried for 2 hours in an oven at 105 °C and cooled in a desiccator for 30 minutes. The salt was weighed out into a clean dry beaker. 0.4121 g of dry sodium chloride was dissolved in approximately 100 ml deionised water in 250 ml volumetric flask and the volume was made up to 250 ml with deionised water. This solution was stored refrigerated in a plastic bottle.

(e)- Working standard solutions (0.0 to 100 mg /l Cl) :

Working standard solutions were prepared by the dilution of the stock solution with deionised water or an appropriate extracting solution. Working standards were stored in plastic bottles.

PROCEDURE :

The manifold (Figure 3.25.) can be used for the determination of chloride in water samples.

Table 3.42. : Reagent flow rate of the final method for the determination of chloride.

Tube	Flow rate* (ml /min)
Sample	0.80
Mercury thiocyanate ferric nitrate reagent	0.42
1.0 M HNO_3	0.16
Wetting agent solution	1.20
Air	0.32
Pull through	1.20

* These flow rates are used for calibration range 0.0 to 100 mg /l Cl.

The solutions can be analysed for chloride using the manifold shown in Figure 3.25. along with standard solutions, blanks and zeros. The samples can be run at the rate of 50 per hour. The colour intensity is measured at 480 nm. Chloride has a linear calibration graph in the ranges 0.0 to 10 and 0.0 to 100 mg /l Cl.

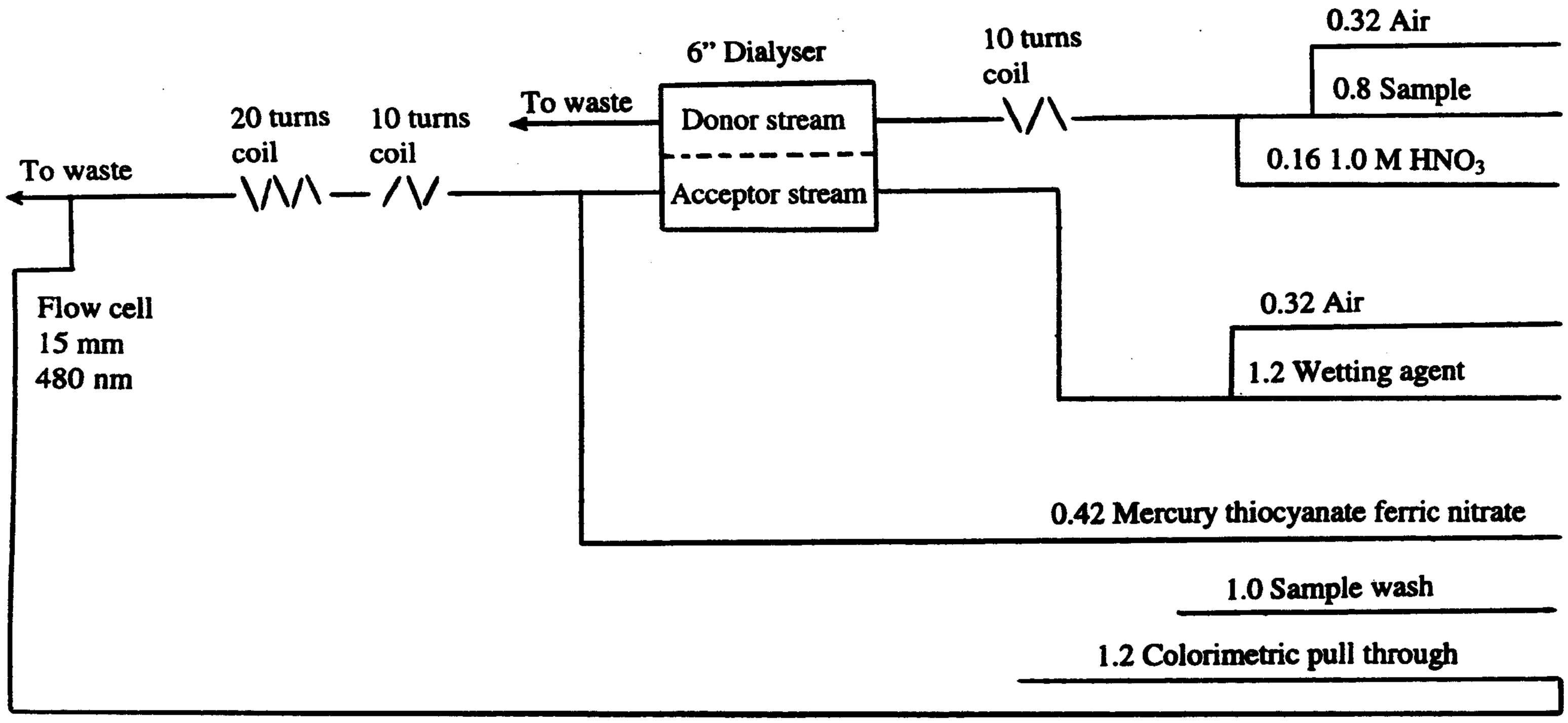


Figure 3.25. : Technicon Autoanalyzer II manifold and the dialysis system of the final method for the determination of chloride.

CHAPTER 4

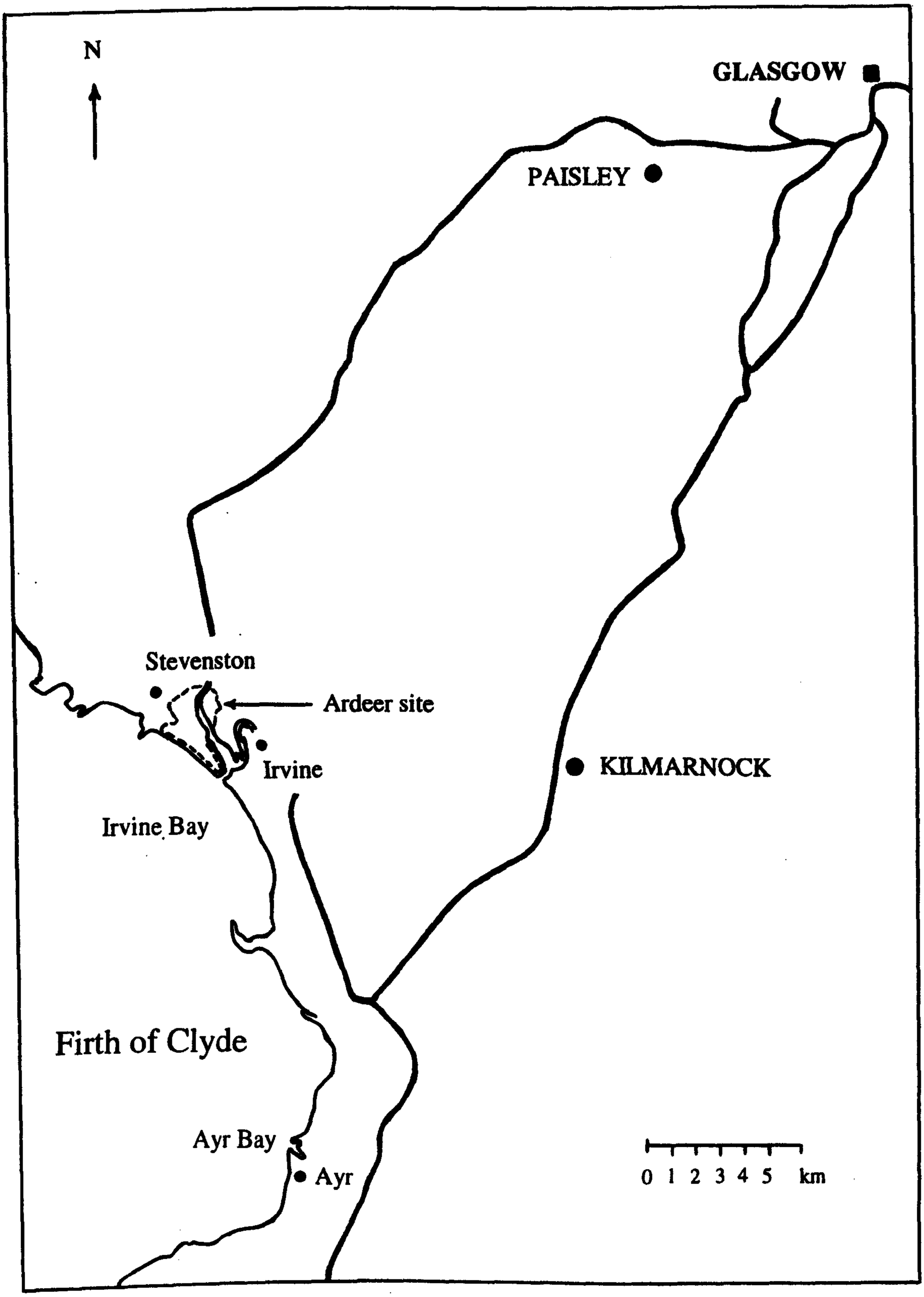
INVESTIGATION OF GROUNDWATER QUALITY AND SOILS IN THE ARDEER SITE

4.1. INTRODUCTION

The Ardeer site of Nobel Enterprises has been used for the manufacture of a range of chemicals and explosives such as Nitro-cellulose, Nitro-glycerine, Dyestuffs and Nylon, and their associated acids, predominately Sulphuric and Nitric acids. It is located at Stevenston, Ayrshire, Scotland (Map 4.1.). A small part of the site was also set aside as a licensed landfill facility. As a result of some of these activities localised contamination has occurred. The particular interest of this study is the nitrogen contamination of the groundwater associated with the areas of the site where the groundwater has been contaminated by ammonium and nitrate.

1971 was the centenary year of the formation of the first British company to manufacture nitro-glycerine-based explosives. Trading under various names and subsequently becoming part of Imperial Chemical Industries Ltd. in 1926, the company reverted to an earlier title of Nobel's Explosives Company Ltd., as a result of re-organisation of a number of ICI divisions in 1971 (Nobel's Explosives Company Limited Report, 1971).

In 1871, Alfred Nobel, backed by a group of Glasgow financiers, began the preparation of the eighth of his seventeen world-wide commercial explosives manufacturing plants at Ardeer on the Ayrshire coast in south west Scotland. The selection of Ardeer as the site for the explosives manufacturing plant was made because the vast expanse of sand dunes provided a barrier of isolation between the process plants and neighbouring towns. The site also offered considerable space for expansion as instanced by the original site of 100 acres growing to the present 2000 acres.



Map 4.1. : The location of the Ardeer site of Nobel Enterprises.

Production began in 1873 with "guhr dynamite", the first safe commercial explosive to incorporate nitro-glycerine. From this beginning evolved a wide range of nitro-glycerine-based explosives carefully formulated for a variety of uses in the mining, quarrying and civil engineering industries.

Nobel's most fundamental invention was the detonator, a short copper tube containing a minute quantity of mercury fulminate that could be handled safely and which could be crimped on to the end of a length of safety fuse to provide a reliable means of detonating nitro-glycerine to full effect even if it was unconfined. Nobel then set about devising a safe method of manufacture for nitro-glycerine (known initially as "blasting oil") and for its use in blasting and mining.

Another of Nobel's basic contributions to explosives technology was the absorption of nitro-glycerine in Keiselguhr, a diatomaceous earth, which could absorb three times its weight of the "blasting oil" : this was the original "guhr dynamite" - first manufactured at Ardeer in 1873. This moist crumbly mass could be cartridged in paper and was the original powder blasting explosive.

A third contribution by Nobel was the formulation and manufacture of "blasting gelatine" formed by gelling 92 % of nitro-glycerine with 8 % of collodion-type nitrocotton. It is the most powerful commercial explosive, and provided a standard of performance against which relative explosive strengths are measured. It was not manufactured at Ardeer on any major scale until 1881 and only small quantities are made, for it is too expensive for most modern purposes.

Nobel also formulated the smokeless powder ballistite, a military propellant patented in 1887. Smokeless powders were part of the general development of weapons which took place in the latter half of the nineteenth century.

Before the end of the nineteenth century, plants were established at Ardeer for the manufacture of soluble nitro-cellulose for gelatine blasting explosives and ballistite; guncotton for manufacture of the Government propellant, Cordite (like ballistite a gelatinised mixture of nitro-cellulose and nitro-glycerine); lead nitrate and picric acid and many other products associated with explosive manufacture. By 1900, the original 100 acres of the Ardeer factory had grown to almost 500 acres and the numbers employed had grown from 50 to 1300.

The passing of the Explosives Act in 1875 to control the manufacture, keeping, conveyance and importation of explosives for legitimate purposes brought the

Ardeer site and the many small explosives factories throughout the country under the control of The Home Office. This regulation of manufacture was only one aspect of government interest in high explosives. The bulk of industrial explosives manufacture in the latter years of the nineteenth century was used in coal mining and, although high explosives gradually replaced gunpowder in British mines, the loss of life in mining accidents was still high.

In 1920, the name was changed to Nobel Industries Limited following more acquisitions, including such famous names as Curtis & Harvey Limited (general explosives business, running back over a century), Kynoch Limited (interests in explosives, ammunition and metals), Eley Brothers Limited (specialists in shotgun cartridges) and Bickford Smith & Co. Limited (safety fuse). At the time of the formation of ICI in 1926, Nobel Industries Limited consisted of 36 subsidiary and 16 associated companies throughout the world and was organised into four groups : explosives, metal and ammunition, artificial leather, collodions and varnish, which formed the nuclei of the Explosives, Metals, Leathercloth and Paints Divisions of ICI.

In 1928, the Ardeer site was still largely concerned in the manufacture of products related closely to those established by Nobel, i.e. blasting explosives derived from nitro-glycerine, propellants, guncotton nitro-cellulose, sulphuric acid and nitric acid, refined glycerine. Between 1928 and the outbreak of the Second World War in 1939, many of the smaller factories inherited from Nobel Industries were closed down, particularly those manufacturing blackpowder, safety fuse and detonators with the transfer of these manufactures to the Ardeer site.

In 1933, the Division Research Department devised a new safety explosive - Hydrox which was a mixture of sodium nitrite and ammonium chloride giving on initiation an evolution of gases from the decomposition of ammonium nitrite. Hydrox itself became obsolete in 1955 but the technique had given rise to the production of useful propellant devices during the 1939 - 1945 War and subsequently to the production of pesticide smoke pellets, a manufacture which was carried on at Ardeer.

In 1934, the manufacture of Cordtex detonating fuse commenced, in which TNT of the original Bickford Cordeau fuse was replaced by PETN (pentaerythritol tetranitrate) and the original lead sheath replaced by textiles. This use of PETN was well in advance of its later use as the base charge in detonators.

In propellant manufacture, the wet-mix method for Cordites, eliminating the use of dry guncotton, led to an improvement in the safety of manufacture. The product was used in anti-aircraft defence rockets and subsequently in engine starter cartridges developed at Ardeer.

The end of 1914 - 1918 War left excess manufacturing capacity for nitro-cellulose. A long programme of work commenced with the development of a safe method of Kiering nitro-cellulose to give low viscosities suitable for industrial application.

The commercial process for the manufacture of methyl methacrylate (Perspex) was originally developed at Ardeer in the thirties, although full scale manufacture was transferred to another ICI site in Billingham.

During the 1939 - 1945 War most of the research effort was involved in work directly connected with military requirements; in particular the development of aeroplane engine starter cartridges, self-heating food cans, "plaster" blasting devices, delay detonators for the RAF, the PIAT bomb (a shaped charge of NE 808 which was insensitive to rifle bullets) and Stokes and Limpets for underwater work on ships.

A new mechanical blasting explosives manufacturing unit was built in 1948 in which the explosives mixing, cartridging and packing operations were improved. A subsequent unit, introduced in 1968, combines the mixing and cartridging processes in one building, remotely controlled, introducing a further degree of safety into the manufacture.

Parallel to the work on the mechanisation of blasting explosives manufacture, work was carried on in the more difficult mechanisation of detonator production. There was, at Ardeer, one unit in which standard detonators were filled and pressed automatically, a series of eight units in which electric detonators were assembled from the three essential items, polyvinyl-chloride covered wire, fuseheads and filled standard detonators, and a series of units in which fusehead assemblies themselves were made automatically. Once again this led to improved safety and better labour utilisation.

Ardeer has also been involved in the production of slurry explosives and of ammonium nitrate/fuel oil mixtures (ANFO) to meet modern blasting techniques. ICI slurry explosives consists of a gelled aqueous solution of ammonium nitrate and sodium nitrate with excess solids held in suspension with the sensitisers. ANFO is simply an intimate mixture of ammonium nitrate (in absorbent prill form) and fuel oil. Both slurry

and ANFO explosives were easy and safe to handle, and had the advantage of completely filling the borehole producing better rock coupling.

The manufacture of chemicals at Ardeer is not new and the production of sulphuric acid, nitric acid and nitro-cellulose began early in the factory's life as essentials of explosives manufacture. As a result of the continual search over in 1950's - 1960's for products to counter the fall in explosives requirements, many other chemical manufactures were established on the Ardeer site. They include isopropyl nitrate, industrial nitro-cellulose including plant to produce plasticised nitro-cellulose granules, (particularly useful in the manufacture of the waterproofing lacquer applied to transparent paper), silicone products (one of the largest manufacturing units in Western Europe) supplied in the form of fluids, resins, greases and elastomers. These silicone products found application as anti-foam agents, anti-stick treatments for paper and baking pans, building sealants, lubricating fluids and greases, electrical resins, medical specialities, cosmetics, mould release agents, polish additives, rubbers for cable insulation and cloth coatings, water repellents for masonry, textiles and leather, adhesive bonding agents and as the active component of the "Flexel" central heating system manufactured and marketed by ICI.

A new organic chemicals complex was erected to produce a range of chemicals based on maleic anhydride. The main uses of maleic anhydride were in unsaturated polyester resins, flame retardant polymers, polyester surface coatings and insecticides. Downstream products of maleic anhydride manufacture include malic acid, fumaric acid, and tetrahydrophthalic anhydride (THPA) used in insecticides, fungicides and in the manufacture of speciality resins. Sanction has been given for the erection of further plants to manufacture tetrahydrofuran (a solvent for elastomeric fibres), butyrolactone (used in the synthesis of Vitamin E), hexahydrophthalic anhydride (a curing agent for epoxy resins), chlorendic acid (a flame retardant) and "Viscofas", a water soluble polymer which may be used in cosmetics, printing inks, and thickening agents for carpet compositions.

In the last 28 years, further rundown in the manufacturing processes at the site took place as a result of the decline in chemical manufacture and the decline in the coal mining industry resulting in a greatly reduced demand for explosives (Tables 4.1. and 4.2.). This decline since the Second World War is shown by the number of

employee at the site (Table 4.3.). The main process now at the site is the manufacture of nitro-cellulose.

Table 4.1. : The decline in coal mining industry in Scotland as shown by the coal production (Pulford, 1999).

Year	Coal production in Scotland
1913	43.2 Mt (peak production) [15 % of UK output].
1989	6.4 Mt [6.0 % of UK output].

Table 4.2. : The decline in coal mining industry in Scotland as shown by the number of coal mines (Pulford, 1999).

Year	Coal mines
1947	250 pits
1993	2 pits

Table 4.3. : The number of the employee in the Ardeer site (Craig, 1999).

Year	Number of employee
1935	6000
1940 - 1945	~ 8000*
1970	4000
1999	300

* Numbers of employee were not published during war time.

This large site with 128 years history of chemical manufacture and contamination is now requiring clean up with the objective of selling or redeveloping parts of the site. This has required a program of soil survey and testing the buildings in the area, prior to knocking down the buildings, removing the rubble, levelling the site, topsoiling and revegetating. Small areas have been redeveloped for small business units or offices. They have also carried out extensive borehole surveys to look for soil contamination.

Groundwater at the site is vulnerable to contamination due to the low CEC, low clay content and high sand content which make the topsoil and subsoil likely to leach contaminants. ICI has carried out an extensive groundwater monitoring program to

identify and trace the groundwater contamination in the site. Many wells were installed for this purpose. They tend to install further boreholes in areas where contamination is found. Therefore, they focused on the landfill area because of the contamination there. Wells No. BH 5, BH 7, BH 9 and BH 10 were installed between the bank of River Garnock and the high water line to check if the landfill area was leaching contaminants to the River Garnock. In August, 1992, a large number of wells (wells No. SW 1 - SW 23 and TW 1 - TW 11) were installed covering the whole site. In December, 1993, a number of wells (wells No. SW 101 - SW 107) were installed covering the Nylon area. Finally, in November, 1995, more wells (wells No. TW 104 - TW 114) were installed to extend cover of the landfill area.

The early surveys showed that groundwater is contaminated. Amin (1995) investigated ammonium contamination of groundwater in the Nylon area. The current study follows the work of Amin (1995) to look at the possibility of bioremediation treatment of the contaminated groundwater in the Nylon area. The bioremediation treatment being considered is by pumping well water to the surface, irrigating the vegetation, and harvesting the vegetation (as vegetation such as ryegrass uses ammonium and nitrate as a fertiliser before it leaches down in soil profile). In the beginning the focus was mainly on ammonium contamination in the Nylon area, but during the course of the current study high levels of ammonium and nitrate were found in the groundwater of other areas. Therefore, the groundwater study was extended away from the Nylon area to the other areas.

In addition , in the current investigation, a soil survey was carried in selected areas where soils have been remediated and vegetated to choose soil samples suitable for experimental work.

Four areas were selected, IOP, H-Acid, Safety Fuse and Nylon and are shown in Map 4.2. The description of each area is given below :

(a)- <u>INTERMEDIATE OXIDATION PLANT (IOP) AREA :</u>

Before the establishment of the IOP plant, the site was occupied by a number of small stores. The bottoming for this building was probably slag from the adjacent former iron works. From 1950 to 1982, the IOP plant used ammonia as raw material and oxidised it to manufacture nitric acid. It was demolished in 1993. The IOP area was

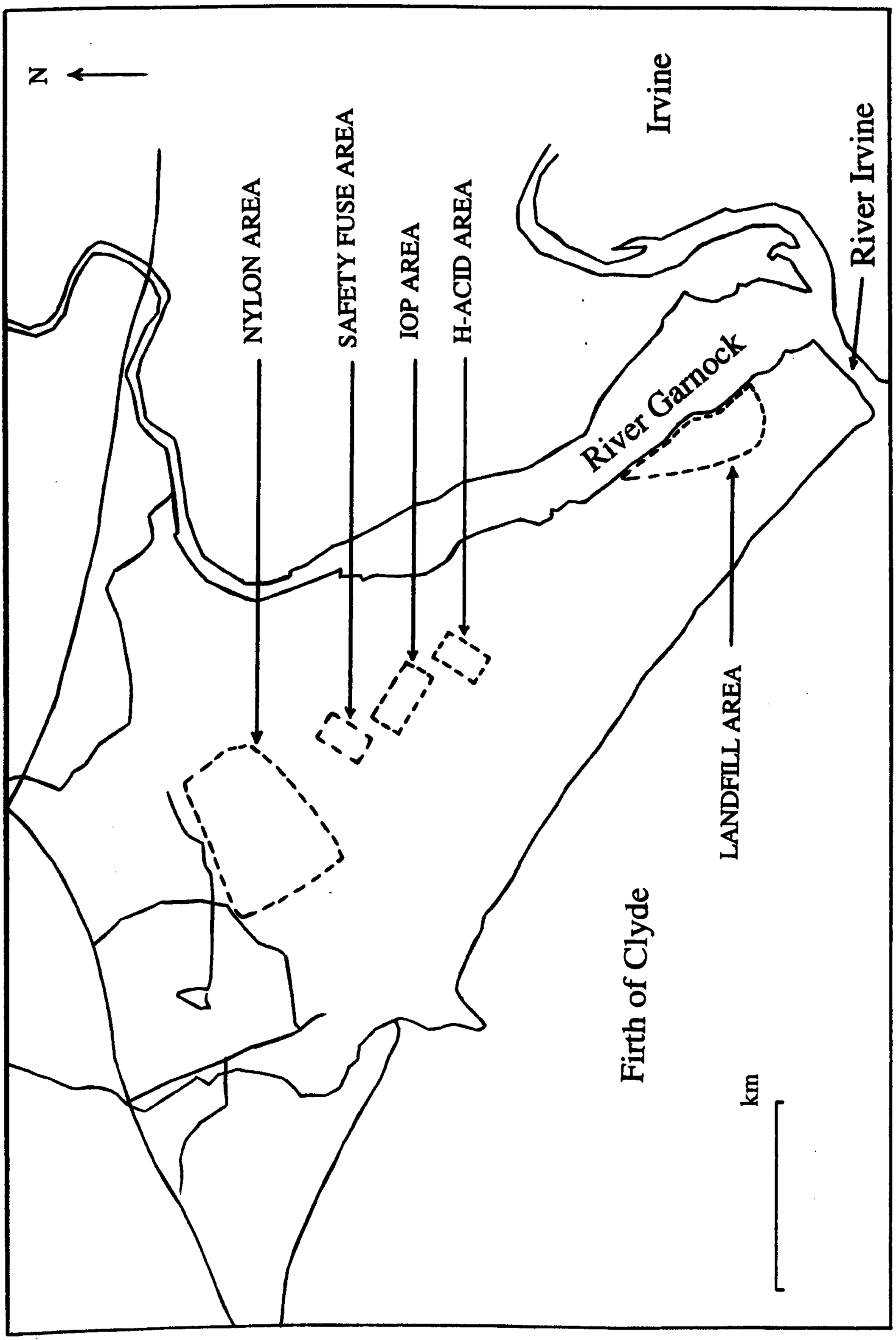
topsoiled to a depth of 15 cm in the Autumn of 1996. The topsoil used was light-textured gravely sandy loam with low organic matter content (6.0 %), friable consistency and granular structure. Soil had a low water holding capacity and free surface drainage. The soil was alkaline with no lime requirement. In addition, the soil had low nitrogen status and there were no other major nutrient deficiencies. Moreover, the soil had a moderate salinity status with no salinity problems.

(b)- <u>H-ACID AREA :</u>

From 1895 to 1970, this area was used for a series of acid mixing station (nitric and sulphuric). The H-Acid plant was constructed in 1971 - 1976, and used for manufacture from 1977 to 1992. The H-Acid plant manufactured Dye Stuff Intermediates (H-Acid) and Monomer One (Bisphenol sulphone). The main products of the H-Acid plant are presented in Figure 4.1. The plant was decontaminated and demolished in 1993 and the area was topsoiled in the Autumn of 1993.

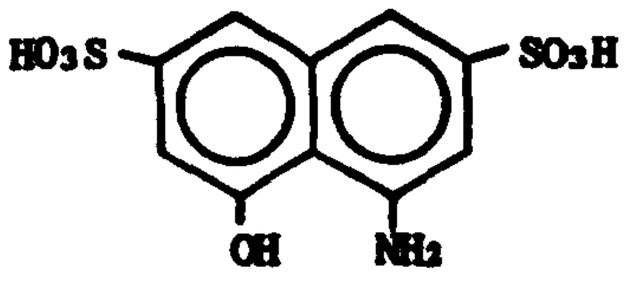
(c)- <u>SAFETY FUSE AREA :</u>

The Safety Fuse plant was built in 1935. It was used for the manufacture of Safety Fuse from 1935 to 1991. Safety Fuse is a detonating cord with a Blackpowder core. The Blackpowder was spun inside a Jute or cotton case, then covered in Bitumen to make it waterproof. The Bitumen was replaced by plastic. Varnish was used as the final covering. In addition, there was a Pesticidal Smoke Generator Plant which used Hexachlorobenzene for the manufacture of pesticidal smoke generators from 1966 to 1990. There were also engineering workshops which used oils/greases and chlorinated solvents to degrease equipment. The plant was decontaminated and demolished in 1995. This area was topsoiled with 15 cm soil in the Autumn of 1996. The topsoil used was the same as that used in the IOP area (Harris, 1999).



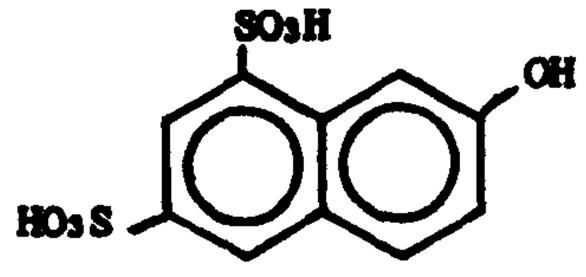
Map 4.2. : The location of IOP, H-Acid, Safety Fuse, Nylon and landfill areas.

STRUCTURAL FORMULA



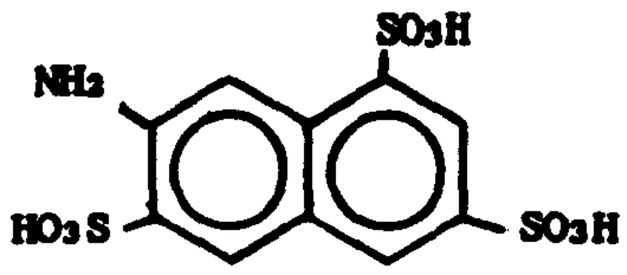
H-Acid

(4-Amino-5-Hydroxynaphthalene-2,7-Disulphonic Acid)



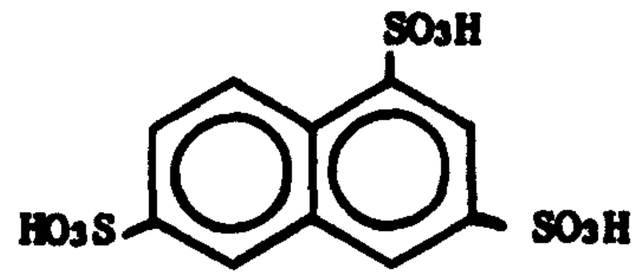
G-Acid

(2-Hydroxynaphthalene-6,8-Disulphonic Acid)



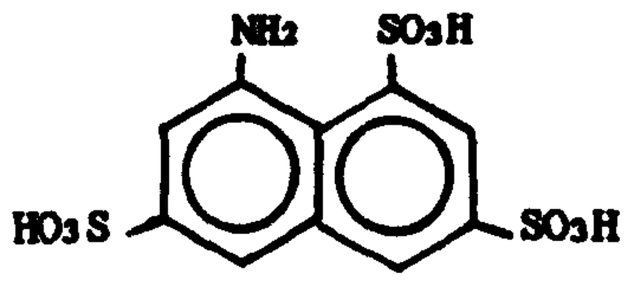
BNTS

(7-Aminonaphthalene-1,3,6-Trisulphonic Acid)



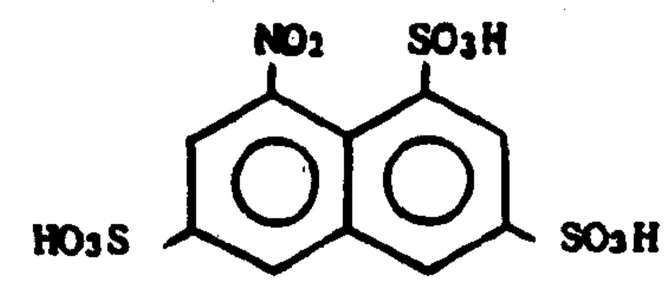
Azoguard

(Naphthalene-1,3,6-Trisulphonic Acid)



Koch Acid

(8-Aminonaphthalene-1,3,6-Trisulphonic Acid)



Nitrokoch Acid

(8-Nitronaphthalene-1,3,6-Trisulphonic Acid)

MOLECULAR FORMULA

$C_{10}H_9S_2NO_7$	-	H-Acid
$C_{10}H_8S_2O_7$	-	G-Acid
$C_{10}H_9S_3NO_9$	-	BNTS
$C_{10}H_8S_3O_9$	-	Azoguard
$C_{10}H_9S_3NO_9$	-	Koch Acid
$C_{10}H_7S_3NO_{11}$	-	Nitrokoch Acid

Figure 4.1. : The main products of the H-Acid plant.

(d)- NYLON AREA :

Before industrial use, up to the early years of this century, this area was a Golf course and Cricket pitch, and in the northern section, was mined for coal. The area north of this area 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 burn. 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 (Amin, 1995). The site buildings were cleared leaving the foundations in place and no further work was done. The main products of the Nylon plant are illustrated in Figure 4.2.

From the groundwater and soil contamination point of view, the major objectives of chapter 4 are :

1- To investigate the distribution and concentration of ammonium-N, nitrate-N and other contaminants such as chloride, potassium, sodium, calcium, magnesium and iron in the groundwater at the Ardeer site.
2- To investigate the sources of contamination and to evaluate the impact of the industrial activity on the groundwater quality at the site.
3- To evaluate the groundwater quality parameters and their seasonal variations over a period of time.
4- To characterise the four selected areas at the site to evaluate the soil contamination due to toxic metals, consequently to select suitable soils for nitrification and plant growth studies.

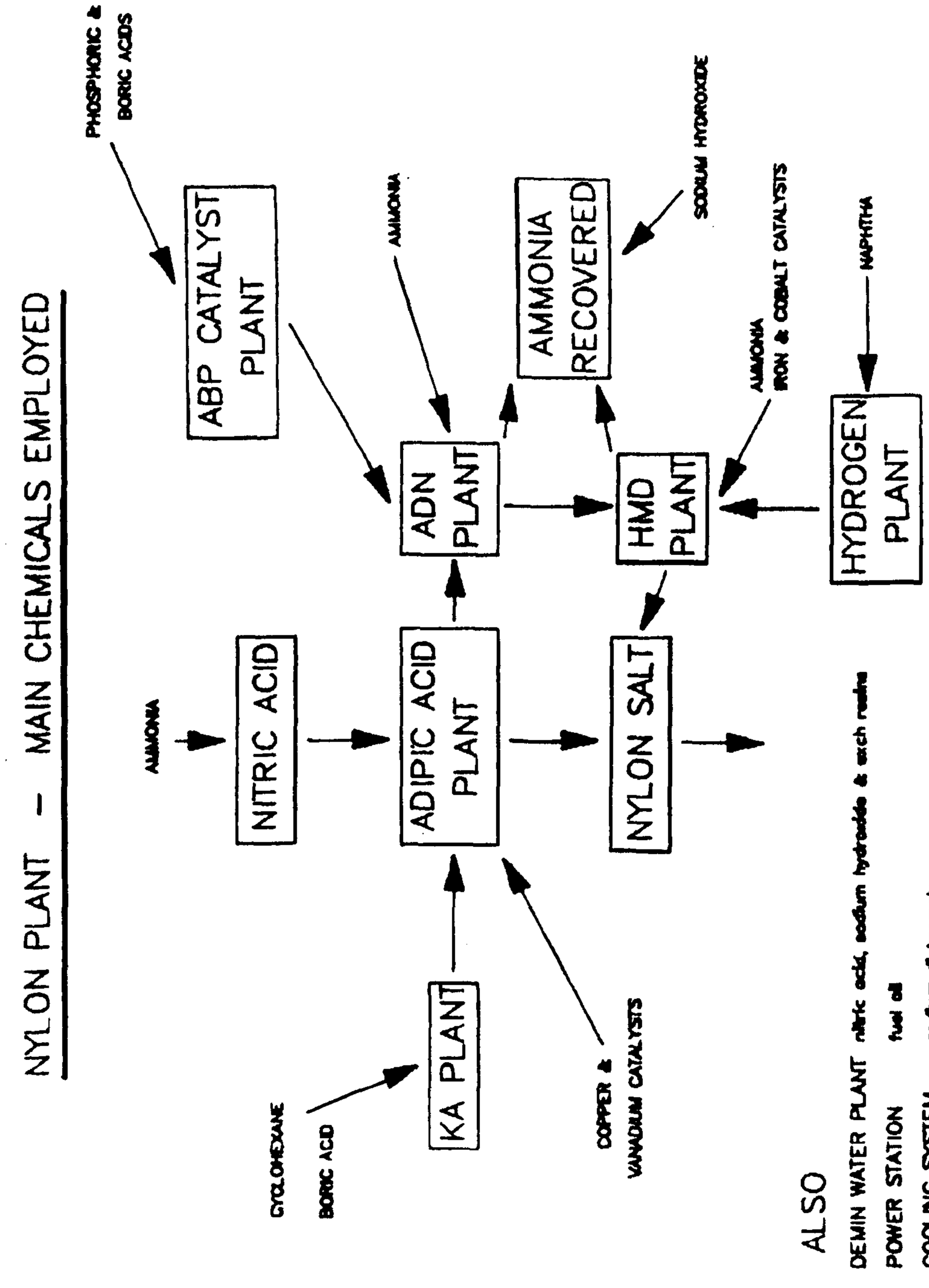


Figure 4.2. : The main products of the Nylon plant.

4.2. MATERIALS AND METHODS

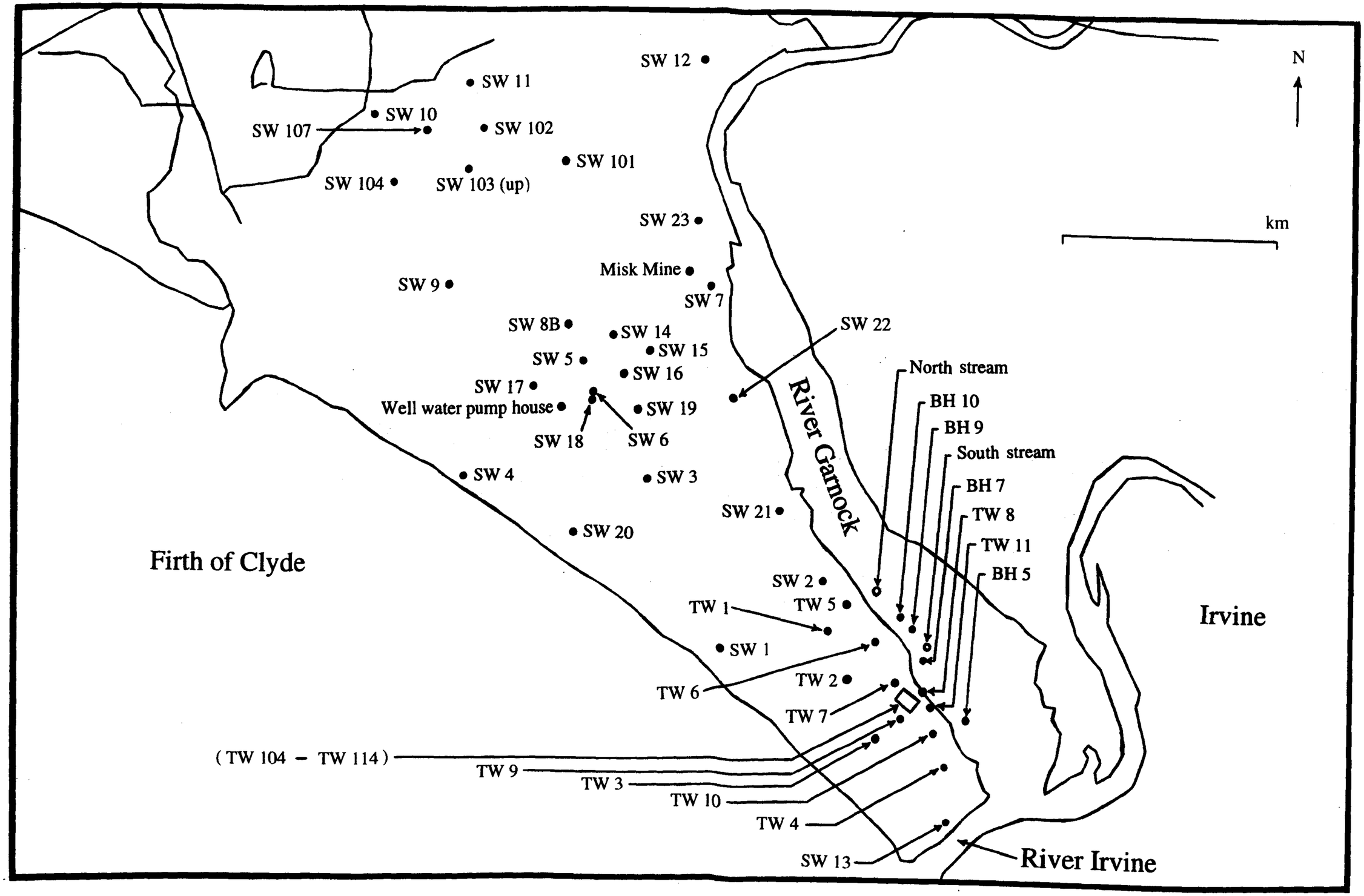
SITE DESCRIPTION :

The Ardeer site of Nobel Enterprises is located at Stevenston, Ayrshire, Scotland. At the northern edge of this site there is a railway line and just across the railway line there is the Master Gott burn. This site has been used for the manufacture of a range of chemicals and explosives such as Nitro-cellulose, Nitro-glycerine, Dyestuffs and Nylon, and their associated acids, predominately Sulphuric and Nitric acids. A small part of the site was also set aside as a licensed landfill facility. The site is shown in Map 4.3.

4.2.1. WATER QUALITY PARAMETERS

4.2.1.1. WATER SAMPLING

The water samples were collected for analysis from the suspected areas. The locations of the sampling points for collecting the water samples are shown in Map 4.3. and illustrated in Tables 4.4. and 4.5.



Map 4.3. : The locations of the sampling points for collecting the water samples from the Ardeer site.

Table 4.4. : Locations of the sampling points of the groundwater monitoring wells.

Well No.	Location
SW 1	Beside Long Sea Pipeline (LSP) [site effluent discharge] pumping station.
SW 2	Wharf road on landward side.
SW 3	Outside Explosive Building in Subfactory "P" (PZ2), through the cutting.
SW 4	North of mix - pak, near shore magazines.
SW 5	Building 400 còmpound.
SW 6	Silver spoon.
SW 7	Cordtex behind Explosive Building in Subfactory "B" (BX 101).
SW 8B	Intermediate Oxidation Plant (IOP) [Nitric acid] across from mining chemicals.
SW 9	Large car park outside gate.
SW 10	Nylon site old tanker area.
SW 11	Nylon site (fuel area).
SW 12	Garnock west between Explosive Building in Subfactory "WP" (WPP2) and Explosive Building in Subfactory "WP" (WPP3).
SW 13	Shore front (Out of gate at point).
SW 14	Organic General Purpose Unit (OGPU) yard.
SW 15	Detonators between Explosive Building Subfactory "T" (TO1) and Explosive Building Subfactory "T" (TO3).
SW 16	In front of the sulphur shed.
SW 17	Between Factory Warehouse/Store (Complex A) and the medical centre.
SW 18	Auto - nitration at Nitro-cellulose (NC) plant.
SW 19	Grassy area at Assembly Building (P500).
SW 20	Shore front beside Factory Magazine (CM12).
SW 21	At Garnock side of Silicones road.
SW 22	Detonators at Explosive Building in Subfactory "D" (DV30).
SW 23	Misk plasting at bottom of Explosive Building in Subfactory "T" (TA12) road.
SW 101	Blackpowder.
SW 102	Nylon site contractors area.
SW 103 (upper)	Nylon site cooling towers.
SW 103 (lower)	Nylon site cooling towers.
SW 104	Nylon site near Ardeer Properties Ltd. (APL) land.
SW 107	Nylon site - North end.
TW 1	Wharf road.
TW 2	Outside mound of Explosive Building in Subfactory "M" (MG6).

Table 4.4. : Continued.

Well No.	Location
TW 3	Near burning station on Wharf road.
TW 4	Short front out of point gate.
Misk Mine*	229135, 640715.
Well water * pump house	Beside Nitro-cellulose (NC) plant.

* Misk Mine and Well water pump house are open shafts with water at ground level, depth unknown.

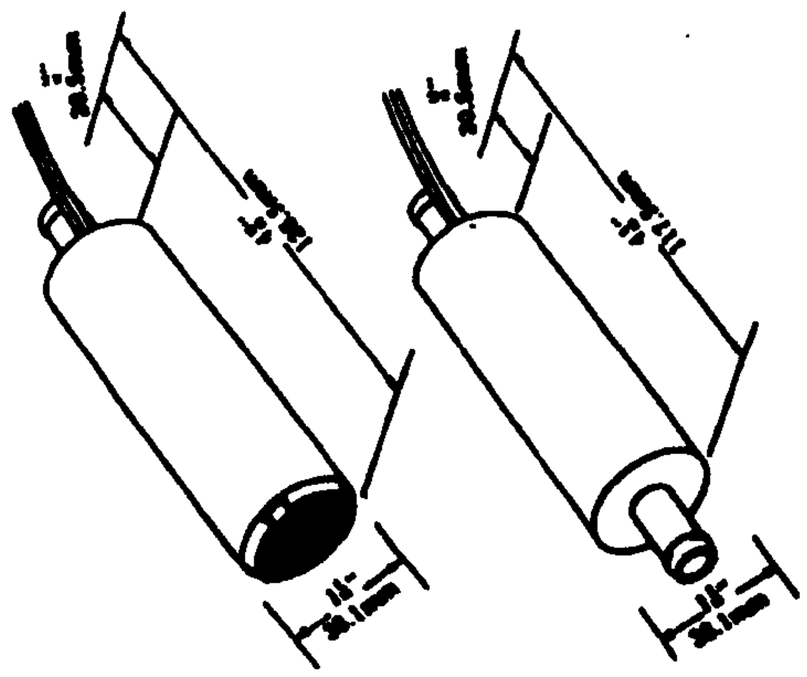
Table 4.5. : Location of the sampling points of the landfill groundwater monitoring wells.

Well No.	Location
TW 5	Landfill area.
TW 6	Landfill area.
TW 7	Landfill area.
TW 8	Landfill area.
TW 9	Landfill area.
TW 10	Landfill area.
TW 11	Landfill area.
TW 104	Landfill area.
TW 105	Landfill area.
TW 106	Landfill area.
TW 107	Landfill area.
TW 108	Landfill area.
TW 109	Landfill area.
TW 110	Landfill area.
TW 111	Landfill area.
TW 112	Landfill area.
TW 113	Landfill area.
TW 114	Landfill area.
BH 5	Landfill area.
BH 7	Landfill area.
BH 9	Landfill area.
BH 10	Landfill area.
North (Silicon) stream*	Landfill area.
South (Wharf) stream*	Landfill area.

* North (Silicon) stream and South (Wharf) stream are streams leaching from the landfill area at ground level.

PROCEDURE FOR SAMPLING THE GROUNDWATER MONITORING WELLS :

The water samples were obtained from 3" inch diameter wells down to a depth of a 20 meters using a submersible pump powered by a 12 volt van battery. An Eijkelkamp Agrisearch Equipment (Van Walt Ltd., Prestwick Lane, Grayswood, Haslemere, Surrey GU27 2DU.) gigant submersible pump was used with a whale submersible in-line pump to obtain the necessary lift (Figure 4.3.).



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12.12.06 SUBMERSIBLE PUMP "GIGANT".
12.12.08 SUBMERSIBLE IN-LINE PUMP "WHALE"

12.12.06  Submersible pump "Gigant",
          ABS/INOX, diam. 36 mm, max.
          capacity 8 l/min, pressure head
          lift 10 m (with final voltage 12
          Volt), with strainer, with 5 m
          PVC coated cable, set of 3 pcs.

12.12.08  Submersible in-line pump "Whale",
          ABS/INOX, diam. 36 mm, max.
          capacity 8 l/min, pressure head
          lift 10 m (with final voltage 12
          Volt), with 0.3 m PVC coated
          cable, set of 3 pcs.
```

Figure 4.3. : The submersible pump "Gigant" and submersible in-line pump "Whale".

The submersible pump was unwound and lowered into the well until it reached the bottom and then it was raised about one metre. The pump outlet was inserted into a waste drum and the pump terminals were connected to the battery and switched on. The required volume of the purge water was pumped out.

The purging was continued until the pH, temperature and conductivity of the water had stabilised (two consecutive values within 10 %). The readings were recorded on the sampling log sheet and the sample bottles were filled.

The pump was switched off and the tubing was removed slowly from the well and the outside was washed with deionised water. Then the pump end was immersed in deionised water and switched on to flush the inside of the pump and tubing. The pump was switched off and disconnected from the battery.

Full decontamination of the equipment was carried out on return to the laboratory as follows :

The pump and tubing were flushed with methanol, rinsed several times with tap water, then rinsed with deionised water. The pump and tubing were drained, dried with paper towel and stored in a polythene bag until required again.

The water samples were stored in the cold room at 2 °C until required for analysis.

4.2.1.2. GROUND WATER SURVEYS

Four surveys were performed for the collection of the water samples for analysis from the suspected areas. The description of each survey is illustrated as follows :

4.2.1.2.1. DESCRIPTION OF THE FIRST SURVEY

This survey was performed in October and November, 1996. The sample description is presented in Table 4.6. The locations of the sampling points are shown in Map 4.3.

Table 4.6. : Groundwater sample description from the first survey.

Well No.	Water level (Depth from ground level in meters)	Total depth of well (meters)	Sampling date
SW 1	2.8	17.0	12 / 11 / 1996
SW 2	2.7	17.0	23 / 10 / 1996
SW 3	1.8	19.0	23 / 10 / 1996
SW 4	4.6	20.0	5 / 11 / 1996
SW 5	3.3	19.0	22 / 11 / 1996
SW 7	1.1	17.0	28 / 10 / 1996
SW 9	3.4	14.0	22 / 11 / 1996
SW 11	4.0	7.0	23 / 10 / 1996
SW 12	3.2	7.0	28 / 10 / 1996
SW 13	3.8	15.0	21 / 10 / 1996
SW 15	1.1	16.0	21 / 11 / 1996
SW 17	3.1	20.0	12 / 11 / 1996
SW 19	1.6	18.0	1 / 11 / 1996
SW 20	3.0	21.0	5 / 11 / 1996
SW 21	1.8	16.0	12 / 11 / 1996
SW 22	1.6	11.0	21 / 10 / 1996
SW 23	1.4	9.0	1 / 11 / 1996
SW 101	1.9	9.0	1 / 11 / 1996
SW 103 (upper)	1.4	11.0	7 / 11 / 1996
SW 103 (lower)	5.5	> 20.0	7 / 11 / 1996
SW 104	1.7	11.0	22 / 11 / 1996
TW 4	3.2	16.0	21 / 10 / 1996
Misk Mine			7 / 11 / 1996
Well water pump house			5 / 11 / 1996

4.2.1.2.2. <u>DESCRIPTION OF THE SECOND SURVEY</u>

This survey was performed in March and April, 1997. The sample description is presented in Table 4.7. The locations of the sampling points are shown in Map 4.3.

Table 4.7. : Groundwater sample description from the second survey.

Well No.	Water level (Depth from ground level in metres)	Total depth of well (metres)	Sampling date
TW 5	3.7	6.0	2 / 4 / 1997
TW 6	4.1	6.0	2 / 4 / 1997
TW 7	4.3	6.0	19 / 3 / 1997
TW 8	3.8	10.0	20 / 3 / 1997
TW 9	3.3	6.0	24 / 3 / 1997
TW 10	2.4	6.0	24 / 3 / 1997
TW 11	2.1	5.0	24 / 3 / 1997
TW 104	4.5	8.0	20 / 3 / 1997
TW 105	4.1	8.0	20 / 3 / 1997
TW 106	3.8	8.0	18 / 3 / 1997
TW 107	4.1	8.0	19 / 3 / 1997
TW 108	4.1	8.0	19 / 3 / 1997
TW 109	4.6	8.0	20 / 3 / 1997
TW 110	4.6	8.0	20 / 3 / 1997
TW 111	4.4	8.0	20 / 3 / 1997
TW 112	3.2	8.0	20 / 3 / 1997
TW 113	4.4	8.0	24 / 3 / 1997
TW 114	5.0	9.0	24 / 3 / 1997
BH 5	0.0	5.0	1 / 4 / 1997
BH 7	0.0	6.0	1 / 4 / 1997
BH 9	0.0	6.0	2 / 4 / 1997
BH 10	0.0	5.0	2 / 4 / 1997
North (Silicon) stream			1 / 4 / 1997
South (Wharf) stream			1 / 4 / 1997

4.2.1.2.3. DESCRIPTION OF THE THIRD SURVEY

This survey was performed in April, May and June, 1997. The sample description is presented in Table 4.8. The locations of the sampling points are shown in Map 4.3.

Table 4.8. : Groundwater sample description from the third survey.

Well No.	Water level (Depth from ground level in metres)	Total depth of well (metres)	Sampling date
SW 1	2.8	17.0	16 / 4 / 1997
SW 2	2.7	17.0	30 / 4 / 1997
SW 3	1.8	19.0	16 / 4 / 1997
SW 4	4.6	20.0	17 / 4 / 1997
SW 5	3.3	19.0	1 / 5 / 1997
SW 6	2.3	8.0	5 / 6 / 1997
SW 7	1.1	17.0	9 / 4 / 1997
SW 8B	1.3	14.0	16 / 5 / 1997
SW 9	3.4	14.0	15 / 5 / 1997
SW 10	3.1	10.0	1 / 5 / 1997
SW 11	4.0	7.0	16 / 4 / 1997
SW 12	3.2	7.0	9 / 4 / 1997
SW 13	3.8	15.0	2 / 5 / 1997
SW 14	1.7	10.0	16 / 5 / 1997
SW 15	1.1	16.0	9 / 4 / 1997
SW 16	2.0	15.0	15 / 5 / 1997
SW 17	3.1	20.0	22 / 5 / 1997
SW 18	2.8	19.0	9 / 6 / 1997
SW 19	1.6	18.0	15 / 5 / 1997
SW 20	3.0	21.0	16 / 5 / 1997
SW 22	1.6	11.0	16 / 4 / 1997
SW 23	1.4	9.0	9 / 4 / 1997
SW 101	1.9	9.0	30 / 4 / 1997
SW 102	2.6	10.0	22 / 4 / 1997
SW 103 (upper)	1.4	11.0	22 / 4 / 1997
SW 103 (lower)	5.5	> 20.0	22 / 4 / 1997
SW 104	1.7	11.0	22 / 4 / 1997
SW 107	1.6	11.0	17 / 4 / 1997
TW 1	1.4	15.0	30 / 4 / 1997
TW 2	3.1	11.0	16 / 5 / 1997
TW 3	1.2	13.0	2 / 5 / 1997
TW 4	3.2	16.0	15 / 5 / 1997
Misk Mine Well water pump house			17 / 4 / 1997 4 / 6 / 1997

4.2.1.2.4. DESCRIPTION OF THE FOURTH SURVEY

This survey was performed in June and August, 1997. The sample description is presented in Table 4.9. The locations of the sampling points are shown in Map 4.3.

Table 4.9. : Groundwater sample description from the fourth survey.

Well No.	Water level (Depth from ground level in metres)	Total depth of well (metres)	Sampling date
SW 2	2.7	17.0	25 / 6 / 1997
SW 3	1.8	19.0	24 / 6 / 1997
SW 4	4.6	20.0	24 / 6 / 1997
SW 5	3.3	19.0	28 / 8 / 1997
SW 8B	1.3	14.0	23 / 6 / 1997
SW 9	3.4	14.0	23 / 6 / 1997
SW 10	3.1	10.0	23 / 6 / 1997
SW 11	4.0	7.0	19 / 8 / 1997
SW 17	3.1	20.0	24 / 6 / 1997
SW 101	1.9	9.0	19 / 8 / 1997
SW 102	2.6	10.0	23 / 6 / 1997
SW 103 (upper)	1.4	11.0	23 / 6 / 1997
SW 103 (lower)	5.5	> 20.0	23 / 6 / 1997
SW 104	1.7	11.0	19 / 8 / 1997
SW 107	1.6	11.0	11 / 8 / 1997
TW 1	1.4	15.0	11 / 8 / 1997
TW 2	3.1	11.0	25 / 6 / 1997
TW 3	1.2	13.0	11 / 8 / 1997

4.2.1.3. CHEMICAL ANALYSIS OF THE GROUNDWATER SAMPLES

The following analysis were carried out :

pH, EC, soluble ions such as NH_4-N, NO_3-N, NO_2-N and Cl and total metals (calcium, magnesium, sodium, potassium and iron).

Some of the water samples contained suspended material. For analysis of the solution ions the samples were filtered through a glass microfibre filter and for the determination of the total metals the samples were digested in HNO_3 acid.

The following water samples needed filtration and digestion :

Wells No. SW 3, SW 5, SW 7, SW 8B, SW 9, SW 10, SW 11, SW 15, SW 16, SW 18, SW 19, SW 22, SW 101, SW 102, SW 103 (lower), SW 104, SW 107, TW 1, TW 2, TW 4, Misk Mine, TW 5, TW 6, TW 8, TW 10, TW 11, TW 104, TW 105, TW 106, TW 109, TW 110, TW 111, TW 112, TW 113, TW 114, BH 5, BH 7, BH 9 and BH 10; and the North (Silicon) stream and South (Wharf) stream.

The remainder of the water samples did not require filtration or digestion.

The determination of NH_4-N, NO_3-N and NO_2-N was the first analysis to be carried out since there may be bacterial activity on these soluble ions if they were left for 1 to 2 weeks without analysis.

(a)- <u>FILTRATION PROCEDURE :</u>

This process was carried out to remove the suspended material from some of the water samples.

The water sample was shaken well and then filtered under vacuum through a 47.0 mm diameter grade GF/C glass microfibre filter (Whatman) and the filtrate was collected in a 28 ml universal bottle. Then the filtrate was transferred to 100 ml plastic bottle for storage.

(b)- <u>DIGESTION PROCEDURE :</u>

This process was carried out to dissolve the undissolved metals such as calcium, magnesium, sodium, potassium and iron which were present in some of the water samples as a precipitate.

The digestion process was carried out in the acid resistant fume cupboard. Large glass funnels (24.5 cm diameter) were inverted over the sample beakers and connected to a water powered vacuum pump to remove HNO_3 acid fumes and the water vacuuming was switched fully on to help in removing the HNO_3 acid fumes.

The water sample was shaken well and then 25 ml was placed in 100 ml beaker, 2.5 ml concentrated HNO_3 acid was added and the contents were swirled by a glass rod. The beaker was placed on a hot plate for 45 minutes at 120 °C to digest and to evaporate about half of the sample volume. The beaker was left to cool to room

temperature and the contents were transferred to a 25 ml volumetric flask with deionised water and the volume was made up to the mark with deionised water. The digested sample was transferred to 60 ml glass bottle for storage. In the case of the determination of sodium and potassium, the digested sample was kept in a plastic bottle since the sodium present in the walls of the glass bottle may be dissolved and contaminate the water sample.

The electrical conductivity (EC) was measured using a Jenway 4070 conductivity meter as in section 2.6.

The pH was measured by a combined glass / reference electrode as in section 2.4.

Ammonium was determined using automated colorimetry with gas phase dialysis as described in section 3.3.2.2.3. using a standard series 0.0 to 10 mg /l NH_4-N in deionised water.

Nitrate was determined using automated colorimetry with liquid liquid dialysis as described in section 3.3.3.2.2. using a standard series 0.0 to 10 mg /l NO_3-N in deionised water.

Nitrite was determined using automated colorimetry with liquid liquid dialysis as described in section 3.3.3.2.2. using a standard series 0.0 to 1.0 mg /l NO_2-N in deionised water.

Chloride was determined using automated colorimetry with liquid liquid dialysis as described in section 3.3.3.2.4. using a standard series 0.0 to 100 mg /l Cl in deionised water.

Potassium was determined by the Flame photometer as described in section 2.11.3. It was determined in the undigested samples using a standard series 0.0 to 5.0 mg K^+ /l in deionised water and in the digested samples using a standard series 0.0 to 5.0 mg K^+ /l in 10 % HNO_3 solution.

Sodium was determined by the Flame photometer as described in section 2.11.4. It was determined in the undigested samples using a standard series 0.0 to 2.5 mg Na^+ /l in deionised water and in the digested samples using a standard series 0.0 to 2.5 mg Na^+ /l in 10 % HNO_3 solution.

Iron was determined by the Atomic Absorption Spectrophotometer as described in section 2.12.1. It was determined in the undigested samples using a

standard series 0.0 to 5.0 mg Fe /l in deionised water and in the digested samples using a standard series 0.0 to 5.0 mg Fe /l in 10 % HNO_3 solution.

Calcium was determined by the Atomic Absorption Spectrophotometer as described in section 2.11.5. It was determined in the undigested samples using a standard series 0.0 to 5.0 mg Ca /l in deionised water containing 10 % vol./vol. of 3 % wt./vol. $SrCl_2.6H_2O$ solution. It was determined in the digested samples using a standard series 0.0 to 5.0 mg Ca /l in 10 % HNO_3 solution containing 10 % vol./vol. of 3 % wt./vol. $SrCl_2.6H_2O$ solution.

Magnesium was determined by the Atomic Absorption Spectrophotometer as described in section 2.11.6. It was determined in the undigested samples using a standard series 0.0 to 0.5 mg Mg /l in deionised containing 10 % vol./vol. of 3 % wt./vol. $SrCl_2.6H_2O$ solution. It was determined in the digested samples using a standard series 0.0 to 0.5 mg Mg /l in 10 % HNO_3 solution containing 10 % vol./vol. of 3 % wt./vol. $SrCl_2.6H_2O$ solution.

4.2.2. CHARACTERISATION OF THE SOILS

4.2.2.1. SOIL SAMPLING

The soil samples were collected from 4 candidate areas for analysis on 5/ 8/ 1997. The profile was cleaned to get definite layers. The soil sample was collected from each layer separately. The soil sample was taken from the bottom layer and up to the top layer to avoid the contamination of the layers in case if the soil sample was taken from the top layer first. Each layer was described according to its contents, texture and colour. The vegetation around each hole was described. The soil samples were brought to the laboratory in labelled plastic bags as soon as possible and kept in the cold room at 2 °C until required for the analysis.

4.2.2.1.1. INTERMEDIATE OXIDATION PLANT (IOP) AREA

Soil samples were collected from 5 sites in the IOP area. The locations of the sampling points are shown in Map 4.4. The sample description is given below :

SITE No. 1 :

Vegetation : White clover and very poor grass (about 50 % coverage).

Depth (cm)

0 - 14 : Topsoil, grey compacted clay.

14 - 24 : Brown compacted and sandier.

> 24 : Building rubble, concrete, brick and sand. Very hard and compacted.

Sample No. 1 a : 0 - 14

Sample No. 1 b : 14 - 24

Sample No. 1 c : > 24

SITE No. 2 :

Vegetation : Predominately white clover and some grass.

Depth (cm)

0 - 12 : Topsoil.

12 - 33 : Soil and ash mixture.

33 - 40 : Ash.

40 - 50 : Sandy, stony subsoil and ash.

Sample No. 2 a : 0 - 12

Sample No. 2 b : 12 - 33

Sample No. 2 c : 33 - 40

Sample No. 2 d : 40 - 50

SITE No. 3 :

Vegetation : White clover and grass (about 78 % coverage, mostly clover).

Depth (cm)

0 - 8 : Topsoil.

> 8 : Soil and ash.

Sample No. 3 a : 0 - 8

SITE No. 4 :

Vegetation : Very poor grass (less than 50 % coverage).

Depth (cm)

0 - 6 : Topsoil and very compacted.

> 6 : Mixture of soil containing stony rubble.

Sample No. 4 a : 0 - 6

SITE No. 5 :

Vegetation : Bare.

Depth (cm)

0 - 15 : Topsoil, sandy.

> 15 : Building rubble.

Sample No. 5 a : 0 - 15

4.2.2.1.2. H-ACID AREA

The soil samples were collected from 4 sites in the H-Acid area. The locations of the sampling points are shown in Map 4.5. The sample description is given below :

SITE No. 6 :

Vegetation : White clover mixed with grass (about 50 % : 50 % mix, full coverage).

Depth (cm)

0 - 17 : Topsoil.

> 17 : Sandy, very stony topsoil and building rubble.

Sample No. 6 a : 0 - 17

SITE No. 7 :

Vegetation : White clover mixed with grass (about 50 % : 50 % mix, full coverage).

Depth (cm)

0 - 11 : Topsoil.

> 11 : Building rubble.

Sample No. 7 a : 0 - 11

SITE No. 8 :

Vegetation : Better than those of sites No. 6 and 7.

Depth (cm)

0 - 22 : Topsoil.

> 22 : Loose sandy soil, very few stones, roots throughout.

Sample No. 8 a : 0 - 22

SITE No. 9 :

Vegetation : White clover mixed with grass (about 50 % : 50 % mix, full coverage).

Depth (cm)

0 - 15 : Topsoil.

> 15 : Sand, concrete and building rubble.

Sample No. 9 a : 0 - 15

Sample No. 9 b : > 15 (most of the stone has been removed from the sample).

4.2.2.1.3. <u>SAFETY FUSE AREA</u>

The soil samples were collected from 4 sites in the Safety Fuse area. The locations of the sampling points are shown in Map 4.6. The sample description is given below :

SITE No. 10 :

Vegetation : White clover and very poor grass (less than 50 % coverage).

Depth (cm)

0 - 23 : Topsoil, sandy and slightly compacted.

23 - 55 : Banded sand.

> 55 : Yellow sand.

Sample No. 10 a : 0 - 23

Sample No. 10 b : 23 - 55

Sample No. 10 c : > 55

SITE No. 11 :

Vegetation : Mostly white clover and poor grass (80 % coverage).

Depth (cm)

0 - 18 : Topsoil, sandy loam.

18 - 40 : Yellow sand.

Sample No. 11 a : 0 - 18

SITE No. 12 :

Vegetation : Predominately white clover with poor grass (50 - 80 % coverage).

Depth (cm)

0 - 20 : Topsoil, compacted.

> 20 : Asphalt rubble.

Sample No. 12 a : 0 - 20

SITE No. 13 :

Vegetation : Very poor grass and very little white clover (less than 50 % coverage).

Depth (cm)

0 - 20 : Topsoil, sandy loam.

20 - 50 : Yellow sand.

Sample No. 13 a : 0 - 20

Sample No. 13 b : 20 - 50

4.2.2.1.4. NYLON AREA

The soil samples were collected from 4 sites in the Nylon area. The locations of the sampling points are shown in Map 4.7. The sample description is given below :

SITE No. 14 :

Vegetation : Thick grass.

Depth (cm)

0 - 30 : Topsoil, sand.

> 30 : Concrete and building rubble.

Sample No. 14 a : 0 - 30

SITE No. 15 :

Vegetation : Grass (gorse and broom).

Depth (cm)

0 - 2 : Mat of litter.

2 - 40 : Disturbed sand.

Sample No. 15 a : 2 - 15

SITE No. 16 :

Vegetation : Very short grass, moss and trefoil.

Depth (cm)

0 -28 : Disturbed sand and stone.

Sample No. 16 a : 0 - 15

SITE No. 17 :

Vegetation : Very short grass, moss, trefoil.

Depth (cm)

0 - 2 : Root and litter mat.

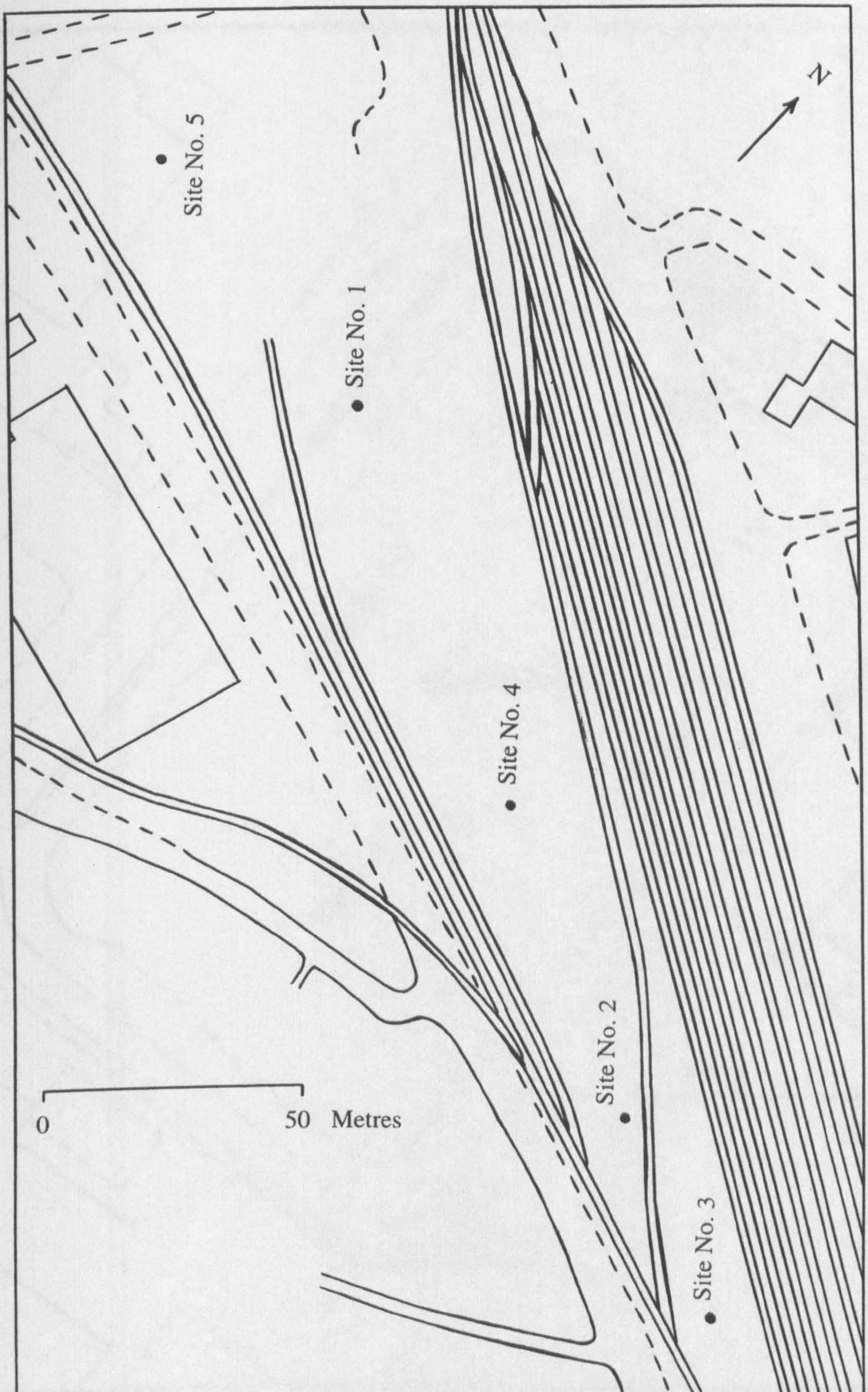
0 - 14 : A-horizon. Naturally developing topsoil. Slightly stony sand.

14 - 47 : Possibly disturbed to this depth.

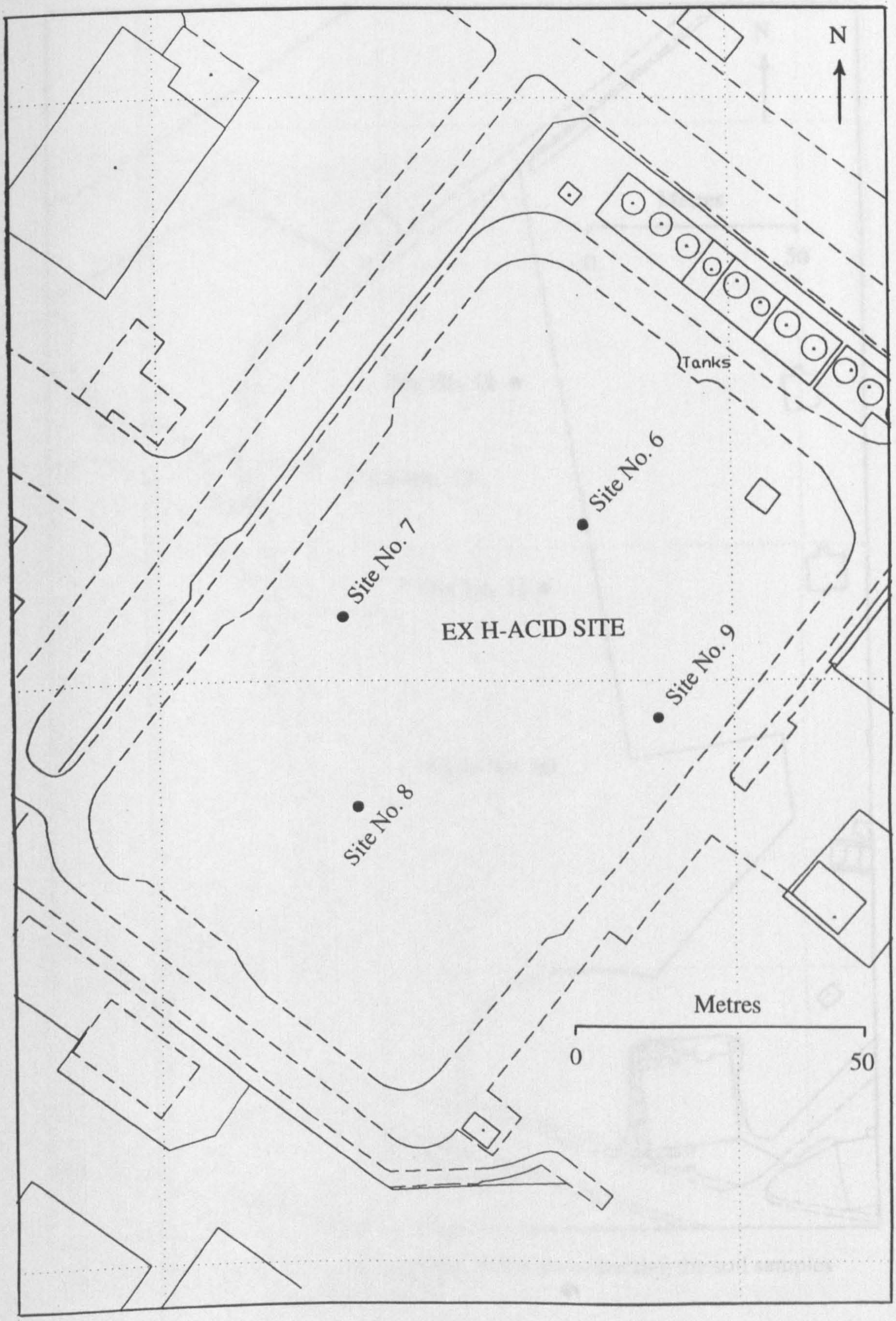
> 47 : Yellow sand.

Sample No. 17 a : 0 - 14

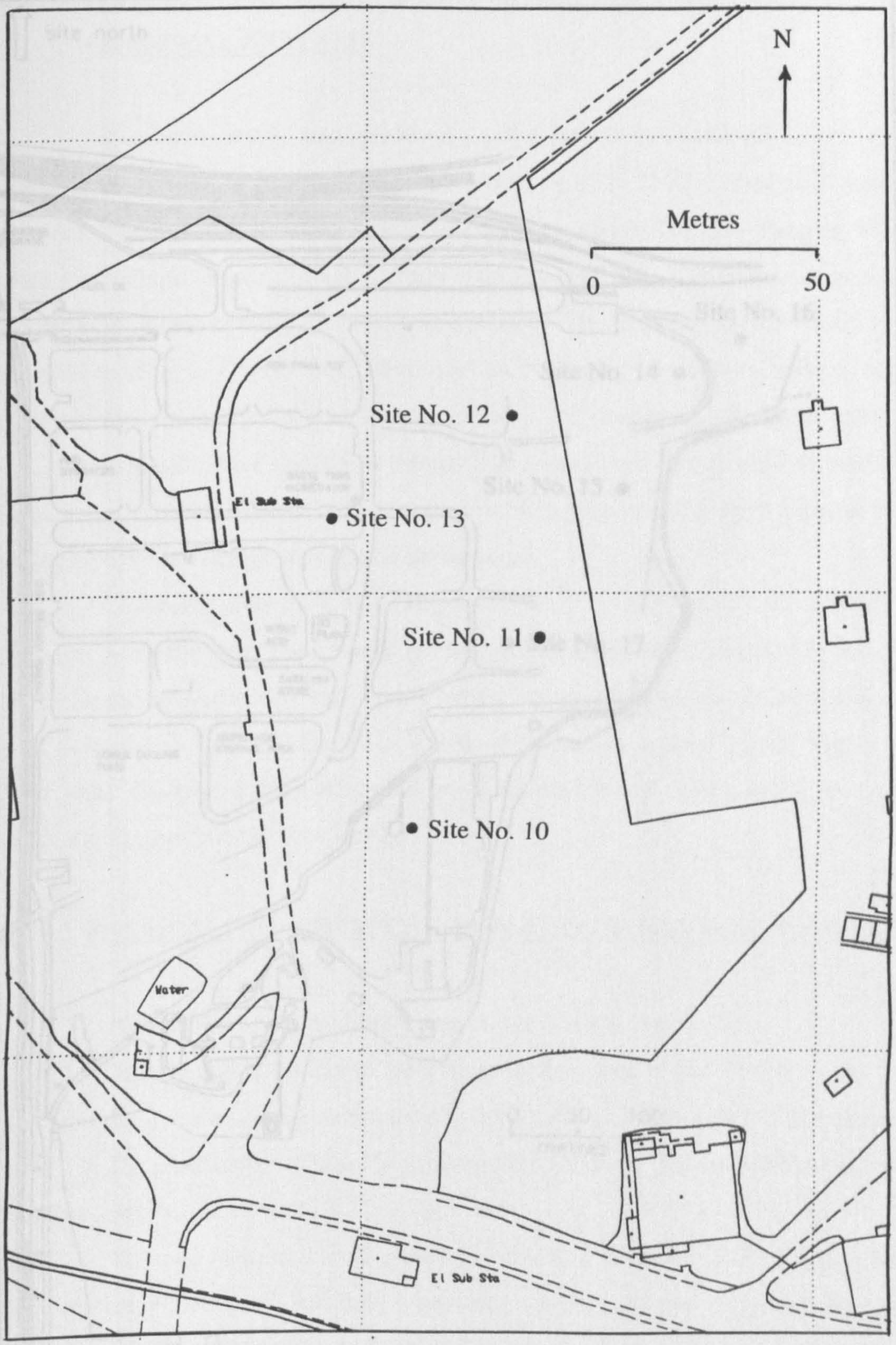
Sample No. 17 b : 14 - 47



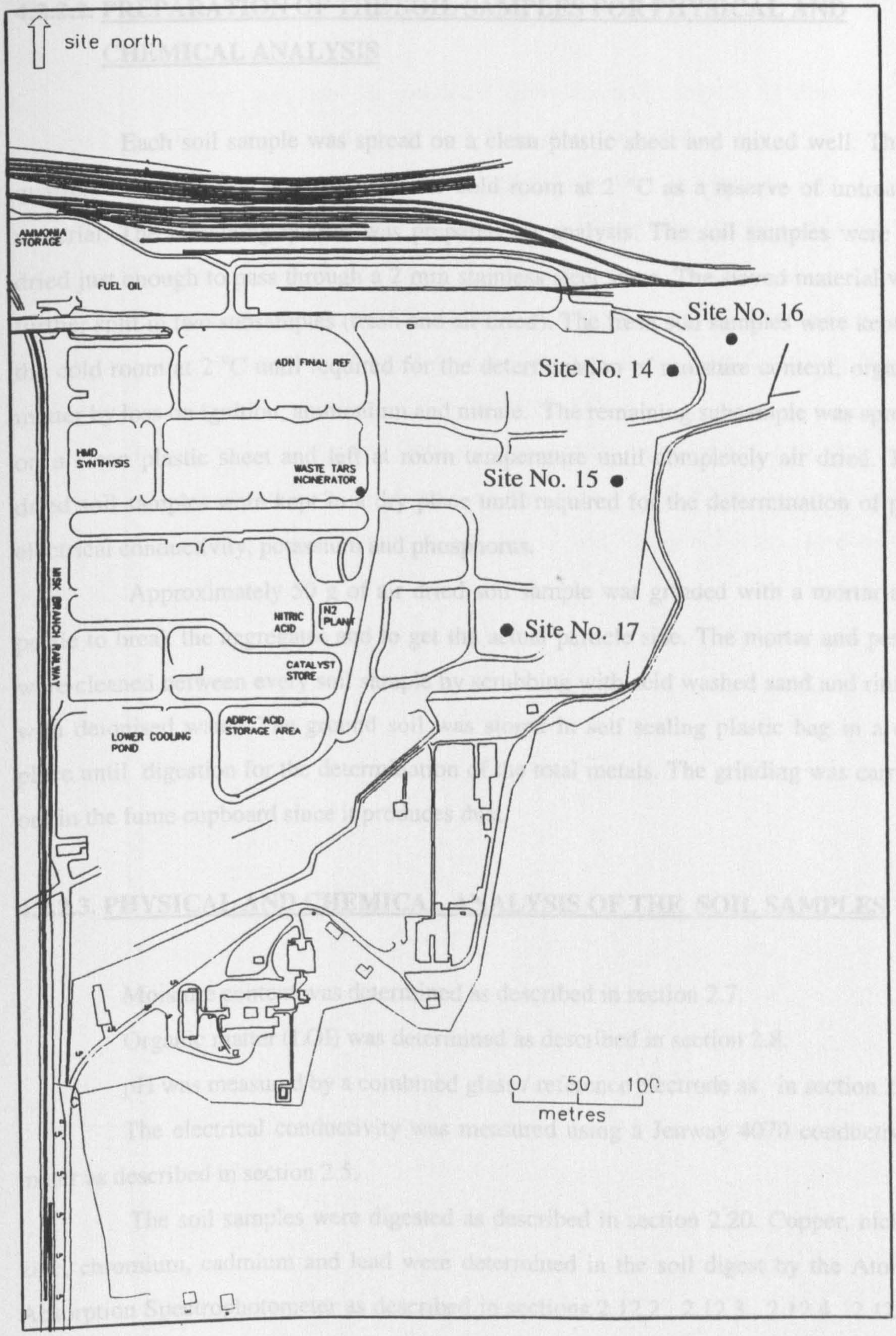
Map 4.4. : The locations of the sampling points for collecting the soil samples from the IOP area.



Map 4.5. : The locations of the sampling points for collecting the soil samples from the H-Acid area.



Map 4.6. : The locations of the sampling points for collecting the soil samples from the Safety Fuse area.



Map 4.7. : The locations of the sampling points for collecting the soil samples from the Nylon area.

4.2.2.2. PREPARATION OF THE SOIL SAMPLES FOR PHYSICAL AND CHEMICAL ANALYSIS

Each soil sample was spread on a clean plastic sheet and mixed well. Three quarters of the sample was stored in the cold room at 2 °C as a reserve of untreated material. The remaining quarter was prepared for analysis. The soil samples were air dried just enough to pass through a 2 mm stainless steel sieve. The sieved material was further split in two subsamples (fresh and air dried). The fresh soil samples were kept in the cold room at 2 °C until required for the determination of moisture content, organic matter by loss on ignition, ammonium and nitrate. The remaining subsample was spread on a clean plastic sheet and left at room temperature until completely air dried. The dried soil samples were kept in a dry place until required for the determination of pH, electrical conductivity, potassium and phosphorus.

Approximately 50 g of air dried soil sample was grinded with a mortar and pestle to break the aggregates and to get the actual particle size. The mortar and pestle were cleaned between every soil sample by scrubbing with acid washed sand and rinsed with deionised water. The ground soil was stored in self sealing plastic bag in a dry place until digestion for the determination of the total metals. The grinding was carried out in the fume cupboard since it produces dust.

4.2.2.3. PHYSICAL AND CHEMICAL ANALYSIS OF THE SOIL SAMPLES

Moisture content was determined as described in section 2.7.

Organic matter (LOI) was determined as described in section 2.8.

pH was measured by a combined glass / reference electrode as in section 2.3.

The electrical conductivity was measured using a Jenway 4070 conductivity meter as described in section 2.5.

The soil samples were digested as described in section 2.20. Copper, nickel, zinc, chromium, cadmium and lead were determined in the soil digest by the Atomic Absorption Spectrophotometer as described in sections 2.12.2., 2.12.3., 2.12.4., 2.12.5., 2.12.6. and 2.12.7.

Cadmium, chromium, nickel, zinc, lead, boron, barium, manganese, magnesium and potassium were determined in the soil digest by the Inductively Coupled

Plasma (ICP) Spectroscopy as described in section 2.14. at Nobel Enterprises, Ardeer site.

Inorganic nitrogen was extracted from the soil samples as described in section 2.17. NH_4-N was determined in the filtrate as described in section 3.1.3.2. using a standard series 0.0 to 1.0 mg /l NH_4-N in 0.5 M K_2SO_4 solution. NO_3-N was determined in the filtrate as described in section 2.11.1.3. using a standard series 0.0 to 1.0 mg /l NO_3-N in 0.5 M K_2SO_4 solution.

Phosphorus was extracted from the soil samples as described in section 2.18. Phosphorus was determined in the neutralised soil extract as described in section 2.11.2. using a standard series 0.0 to 1.0 mg P /l in 0.5 M Na_2SO_4.

Potassium was extracted from the soil samples as described in section 2.19. Potassium was determined in the filtrate by the Flame photometer as described in section 2.11.3. using a standard series 0.0, 5, 10, 15 and 20 mg K^+ /l in 1 M NH_4NO_3 solution.

4.3. RESULTS AND DISCUSSION

4.3.1. WATER QUALITY PARAMETERS

4.3.1.1. pH

The seasonal variations of the pH in the groundwater monitoring wells are presented in Table 4.10. The chemical composition of the landfill groundwater monitoring wells (Spring, 1997) is shown in Table 4.11. As can be seen, the majority of the wells had pH in the range from 6.1 to 8.3 but some wells were acidic (pH below 4.3). The North stream had a slightly higher pH (7.7) and the South stream had an acidic pH (3.3). The high pH in the Nylon area could be due to ammonia spillage in the Nylon area since ammonia was used in the nylon production plant and an associated nitric acid plant and this is evidenced by the high pH of wells No. SW 10, SW 104, SW 102, SW 103 (upper) and SW 107 [pH in the range from 7.3 - 8.4]. The acidic pH could be due to acid leakage as use of strong acids was widespread at the site.

In the majority of the wells, there were no great seasonal variations of the pH except wells No. SW 3 and TW 2 which had a variable pH.

4.3.1.2. AMMONIUM-N

The seasonal variations of ammonium-N in the groundwater monitoring wells are given in Table 4.12. The chemical composition of the landfill groundwater monitoring wells (Spring, 1997) is shown in Table 4.11. It was noticed that the natural background level of ammonium was < 1.0 mg NH_4-N /l at the site. About one third of the wells were contaminated with ammonium particularly wells No. SW 11, SW 14, SW 16, SW 107, TW 8, TW 104, TW 105, TW 106, TW 112 and TW 114. Well No. SW 15 was extremely contaminated. The North stream was not contaminated with ammonium but the South stream was contaminated.

There were three areas (hot spots) at the site with ammonium contamination, these areas were quite localised. In the first hot spot, one well (well No. SW 14) had a moderate level of ammonium, one well (well No. SW 16) had high level of ammonium and the main well was well No. SW 15 which was enormously contaminated with ammonium. However, there were a number of wells (wells No. SW 14, SW 5, SW 6,

SW 8B, SW 17 - SW 19 and well water pump house) which are close to it and were not high in ammonium. Ammonium level increases with the depth of the wells (see Tables 4.7. and 4.8.) of the first hot spot, particularly in wells No. SW 15 and SW 16. In addition, the high and very high levels of ammonium occurred in wells with higher water levels in the range from 1.1 - 2.0 metres below ground surface (see Table 4.8.) and this may be contribute to the ammonium level in the drainage water. In the second hot spot, in the Nylon area, there were wells No. SW 11 and SW 107. The high levels of ammonium in these wells may be due to ammonia spillage in the Nylon area since ammonia was used in the nylon production plant and an associated nitric acid. In the third hot spot, wells No. TW 8, TW 104 - TW 106, TW 112 and TW 114 showed contamination.

In the majority of the wells, there were no great seasonal variations of ammonium level except wells No. SW 11, SW 15 and SW 107 which had variable levels of ammonium.

4.3.1.3. NITRATE-N

The seasonal variations of nitrate-N in the groundwater monitoring wells are summarised in Table 4.13. The chemical composition of the landfill groundwater monitoring wells (Spring, 1997) is shown in Table 4.11. It was observed that the natural background level of nitrate was < 1.0 mg NO_3-N /l at the site. About half of the wells were contaminated with nitrate particularly wells No. SW 2, SW 3, SW 6, SW 11, SW 23, TW 2, BH 7 and BH 9. Wells No. SW 14 - SW 16 and SW 18 were extremely contaminated. The North stream was contaminated with nitrate but the South stream was not contaminated.

There were two hot spots of nitrate at the site, the first hot spot includes wells No. SW 3, SW 6, SW 14 - SW 16 and SW 18. The second hot spot includes wells No. SW 2, TW 2, BH 7 and BH 9; and the North stream. The high nitrate levels could be attributed to the spillage of nitric acid in the H-Acid area as evidenced by the high levels of nitrate with low pH in wells No. SW 6, SW 14, SW 16 and SW 18. The nitrate contamination was very closely localised. Although well No. SW 18 is very close to well No. SW 6, it had a level of nitrate higher than that of well No. SW 6 and this could be because well No. SW 18 is deeper than well No. SW 6 (see Table 4.8.). By

comparing nitrate concentration in well No. SW 6 with that of well No. SW 18, there is some evidence that nitrate increases with the depth of the well in the hot spot, particularly in wells No. SW 15, SW 16 and SW 18 (see Table 4.8.). Furthermore, the high and very high levels of nitrate occurred in wells with high water levels in the range from 1.1 - 2.8 metres below ground surface (see Table 4.8.).

Of the all wells, there were more seasonal variations of nitrate level in about half of the wells particularly in wells No. SW 2 and SW 3 which had very variable levels of nitrate and in wells No. SW 15 and SW 23 which had variable levels of nitrate. Ammonium and nitrate were not necessarily found together as can be seen from wells No. SW 6, SW 14, SW 18, SW 23 and TW 2.

4.3.1.4. NITRITE-N

The seasonal variations of nitrite-N in the groundwater monitoring wells are demonstrated in Table 4.14. The chemical composition of the landfill groundwater monitoring wells (Spring, 1997) is shown in Table 4.11. As can be seen, the natural background level of nitrite was < 0.1 mg NO_2-N /l at the site. Wells No. SW 11 and SW 18 were contaminated with nitrite. The North and South streams were not contaminated with nitrite. Nitrite is unstable under acid conditions and would not be expected in acidic groundwaters.

Regarding the seasonal variations of nitrite level, well No. SW 11 had a very variable level of nitrite.

Table 4.10. : Seasonal variations of the pH in the groundwater monitoring wells.

Well No.	Autumn (1996)	Spring (1997)	Summer (1997)
SW 1	7.2	7.7	----
SW 2	1.9	1.4	1.6
SW 3	4.3	7.9	7.6
SW 4	7.0	7.8	7.6
SW 5	7.0	7.2	7.6
SW 6	----	2.1	----
SW 7	7.1	7.9	----
SW 8B	----	4.1	4.4
SW 9	7.1	8.2	8.0
SW 10	----	8.3	8.0
SW 11	6.7	6.5	5.1
SW 12	6.8	6.5	----
SW 13	7.4	7.3	----
SW 14	----	1.0	----
SW 15	7.2	7.6	----
SW 16	----	3.8	----
SW 17	7.6	7.8	7.8
SW 18	----	3.7	----
SW 19	7.6	8.3	----
SW 20	7.6	8.0	----
SW 21	7.5	----	----
SW 22	7.5	8.2	----
SW 23	7.4	7.9	----
SW 101	7.2	7.4	7.5
SW 102	----	8.0	7.7
SW 103 (upper)	7.5	7.7	7.5
SW 103 (lower)	7.9	8.5	8.1
SW 104	7.3	8.2	8.1
SW 107	----	8.4	8.1
TW 1	----	7.7	7.8
TW 2	----	6.2	4.1
TW 3	----	7.6	7.7
TW 4	7.5	8.4	----
Misk Mine	7.8	8.5	----
Well water pump house	7.9	8.1	----

---- No sample collected.

Table 4.11. : Chemical composition of the landfill groundwater monitoring wells (Spring, 1997).

Well No.	pH	EC* (ms /cm)	NH_4-N (mg /l)	NO_3-N (mg /l)	NO_2-N (mg /l)	Cl (mg /l)
TW 5	2.4	4.3	1.3	4.7	< 0.1	54
TW 6	8.1	1.1	< 0.1	< 0.1	< 0.1	54
TW 7	2.1	4.1	0.3	0.5	< 0.1	678
TW 8	7.5	5.2	41.0	0.2	< 0.1	1050
TW 9	8.2	1.0	< 0.1	0.2	< 0.1	54
TW 10	8.3	1.2	< 0.1	7.7	< 0.1	141
TW 11	8.2	3.0	< 0.1	8.0	< 0.1	278
TW 104	2.4	4.4	21.0	0.1	< 0.1	680
TW 105	2.3	4.8	17.0	0.1	< 0.1	1065
TW 106	2.3	7.7	58	4.0	< 0.1	2208
TW 107	2.0	5.0	0.9	< 0.1	< 0.1	738
TW 108	1.5	15.0	1.9	2.9	< 0.1	592
TW 109	8.2	2.9	< 0.1	8.0	< 0.1	332
TW 110	7.6	2.1	5.4	0.6	< 0.1	295
TW 111	8.2	1.3	4.7	< 0.1	< 0.1	151
TW 112	2.3	5.0	19.3	0.8	< 0.1	956
TW 113	8.1	2.1	< 0.1	2.1	< 0.1	109
TW 114	2.7	2.9	38.0	0.3	< 0.1	142
BH 5	8.1	5.9	< 0.1	0.1	< 0.1	1997
BH 7	7.2	36.0	< 0.1	31.0	< 0.1	10414
BH 9	7.5	36.0	< 0.1	14.0	< 0.1	9855
BH 10	7.9	13.3	< 0.1	< 0.1	< 0.1	4827
North (Silicon) stream	7.7	45.0	< 0.1	12.0	< 0.1	13122
South (Wharf) stream	3.3	19.0	9.4	0.3	< 0.1	7468

* EC = Electrical conductivity.

Table 4.11. : Continued.

Well No.	K (mg /l)	Na (mg /l)	Ca (mg /l)	Mg (mg /l)	Fe (mg /l)
TW 5	2.5	41.3	460	104	506
TW 6	10.0	78	169	22.8	123
TW 7	40.0	46.3	178	16.0	34.5
TW 8	60	143	720	524	147
TW 9	35.0	133	73	6.6	< 0.05
TW 10	15.0	103	104	37.2	0.1
TW 11	165	425	305	83	154
TW 104	275	188	190	106	290
TW 105	215	225	210	93	208
TW 106	110	115	435	208	462
TW 107	15.0	70	165	24.7	82
TW 108	20.0	90	190	40.2	235
TW 109	82	425	241	132	4.7
TW 110	42.5	169	227	69	24.5
TW 111	12.0	133	111	26.2	89
TW 112	100	103	221	177	335
TW 113	170	155	190	144	0.2
TW 114	20.0	109	234	79	124
BH 5	55	900	122	399	3.4
BH 7	180	5625	327	684	34.0
BH 9	210	5025	239	786	31.0
BH 10	115	3150	90	463	2.0
North (Silicon) stream	250	7100	265	896	0.9
South (Wharf) stream	185	2950	296	564	76

Table 4.12. : Seasonal variations of ammonium-N (mg /l) in the groundwater monitoring wells.

Well No.	Autumn (1996)	Spring (1997)	Summer (1997)
SW 1	< 0.1	< 0.1	----
SW 2	3.4	3.9	2.2
SW 3	< 0.1	< 0.1	< 0.1
SW 4	< 0.1	< 0.1	< 0.1
SW 5	< 0.1	< 0.1	<0.1
SW 6	----	0.7	----
SW 7	< 0.1	< 0.1	----
SW 8B	----	2.0	1.9
SW 9	< 0.1	< 0.1	< 0.1
SW 10	----	< 0.1	< 0.1
SW 11	37.9	28.0	9.3
SW 12	< 0.1	< 0.1	----
SW 13	< 0.1	< 0.1	----
SW 14	----	17.8	----
SW 15	7230	8090	----
SW 16	----	117	----
SW 17	< 0.1	< 0.1	< 0.1
SW 18	----	5.1	----
SW 19	< 0.1	< 0.1	----
SW 20	< 0.1	< 0.1	----
SW 21	< 0.1	----	----
SW 22	< 0.1	< 0.1	----
SW 23	< 0.1	< 0.1	----
SW 101	< 0.1	< 0.1	< 0.1
SW 102	----	< 0.1	< 0.1
SW 103 (upper)	< 0.1	< 0.1	< 0.1
SW 103 (lower)	< 0.1	< 0.1	< 0.1
SW 104	< 0.1	< 0.1	< 0.1
SW 107	----	4.3	17.8
TW 1	----	< 0.1	< 0.1
TW 2	----	1.3	5.0
TW 3	----	< 0.1	< 0.1
TW 4	< 0.1	< 0.1	----
Misk Mine	< 0.1	< 0.1	----
Well water pump house	< 0.1	< 0.1	----

---- No sample collected.

Table 4.13. : Seasonal variations of nitrate-N (mg /l) in the groundwater monitoring wells.

Well No.	Autumn (1996)	Spring (1997)	Summer (1997)
SW 1	2.4	1.9	----
SW 2	39.0	0.2	0.1
SW 3	23.0	< 0.1	0.0
SW 4	< 0.1	< 0.1	< 0.1
SW 5	8.8	6.7	7.1
SW 6	----	121	----
SW 7	5.7	2.9	----
SW 8B	----	9.0	9.8
SW 9	< 0.1	< 0.1	< 0.1
SW 10	----	6.9	7.3
SW 11	42.7	24.0	45.5
SW 12	0.3	2.0	----
SW 13	1.8	1.3	----
SW 14	----	3919	----
SW 15	10860	8099	----
SW 16	----	840	----
SW 17	0.5	3.1	3.6
SW 18	----	2701	----
SW 19	1.6	< 0.1	----
SW 20	< 0.1	< 0.1	----
SW 21	< 0.1	----	----
SW 22	< 0.1	2.2	----
SW 23	231	133	----
SW 101	3.1	0.3	< 0.1
SW 102	----	0.7	1.1
SW 103 (upper)	< 0.1	< 0.1	< 0.1
SW 103 (lower)	< 0.1	< 0.1	< 0.1
SW 104	1.7	2.2	< 0.1
SW 107	----	1.4	0.5
TW 1	----	< 0.1	< 0.1
TW 2	----	17.0	18.2
TW 3	----	0.3	< 0.1
TW 4	2.8	3.3	----
Misk Mine	1.4	0.2	----
Well water pump house	0.9	0.3	----

---- No sample collected.

Table 4.14. : Seasonal variations of nitrite-N (mg /l) in the groundwater monitoring wells.

Well No.	Autumn (1996)	Spring (1997)	Summer (1997)
SW 1	< 0.1	< 0.1	----
SW 2	< 0.1	< 0.1	< 0.1
SW 3	< 0.1	< 0.1	< 0.1
SW 4	< 0.1	< 0.1	< 0.1
SW 5	< 0.1	< 0.1	< 0.1
SW 6	----	0.1	----
SW 7	< 0.1	< 0.1	----
SW 8B	----	< 0.1	< 0.1
SW 9	< 0.1	< 0.1	< 0.1
SW 10	----	< 0.1	< 0.1
SW 11	3.0	52	< 0.1
SW 12	< 0.1	< 0.1	----
SW 13	< 0.1	< 0.1	----
SW 14	----	1.0	----
SW 15	< 0.1	1.0	----
SW 16	----	0.2	----
SW 17	< 0.1	< 0.1	< 0.1
SW 18	----	9.0	----
SW 19	< 0.1	< 0.1	----
SW 20	< 0.1	< 0.1	----
SW 21	0.4	----	----
SW 22	< 0.1	< 0.1	----
SW 23	0.1	0.2	----
SW 101	< 0.1	< 0.1	< 0.1
SW 102	----	< 0.1	< 0.1
SW 103 (upper)	< 0.1	< 0.1	< 0.1
SW 103 (lower)	< 0.1	< 0.1	< 0.1
SW 104	< 0.1	< 0.1	< 0.1
SW 107	----	< 0.1	< 0.1
TW 1	----	< 0.1	< 0.1
TW 2	----	< 0.1	< 0.1
TW 3	----	< 0.1	< 0.1
TW 4	< 0.1	< 0.1	----
Misk Mine	< 0.1	< 0.1	----
Well water pump house	< 0.1	< 0.1	----

---- No sample collected.

4.3.1.5. CHLORIDE

The seasonal variations of chloride in the groundwater monitoring wells are presented in Table 4.15. The chemical composition of the landfill groundwater monitoring wells (Spring, 1997) is shown in Table 4.11. As can be seen, the wells were quite variable in chloride. The natural background level of chloride was < 50 mg Cl /l at the site. More than one third of the wells was contaminated with chloride. Wells No. SW 5, SW 13, SW 15, TW 3, TW 4, Misk Mine, TW 7, TW 8, TW 104 - TW 108, TW 112, BH 5, BH 7, BH 9 and BH 10 were extremely contaminated. The North and South streams were both extremely contaminated.

There were two areas with exceptionally high levels of chloride at the site. The first area includes wells No. SW 13, TW 1 - TW 4, TW 7, TW 8, TW 10, TW 11, TW 104 - TW 109, TW 111 - TW 114, BH 5, BH 7, BH 9 and BH 10; and the North and South streams. The second area includes wells No. SW 5, SW 14 - SW 17, and well water pump house. Chloride level increases with the depth of the wells (total depth in the range from 13 - > 20 metres) [see Table 4.8.]. In addition, the high and very high levels of chloride occurred in wells with lower water levels ranged from 3.2 - 5.0 metres below ground surface (see Table 4.7.). Chloride contamination was widespread at the site due to its excessive solubility.

There were no great seasonal variations of chloride level in the majority of the wells except wells No. SW 3, SW 17, SW 103 (upper), SW 103 (lower), TW 2, TW 3 and TW 4 had variable levels of chloride.

4.3.1.6. SODIUM

The seasonal variations of sodium in the groundwater monitoring wells are presented in Table 4.16. The chemical composition of the landfill groundwater monitoring wells (Spring, 1997) is shown in Table 4.11. It was noticed that the natural background level of sodium was < 50 mg Na /l at the site. About half of the wells was contaminated with sodium. Wells No. SW 5, SW 13, SW 15, SW 16, SW 18, SW 23, TW 3, TW 4, BH 5, BH 7, BH 9 and BH 10 were extremely contaminated. The North and South streams were extremely contaminated.

Sodium level was the same as chloride level for wells with very high levels of sodium (wells No. SW 13, TW 2, and TW 3) since they were close to the coast of the sea (Firth of Clyde). Sodium level in wells No. SW 23, SW 16, SW 18 and SW 15 does not match with chloride level of the same wells and this may be attributed to the very high levels of nitrate in wells No. SW 15, SW 16 and SW 18; and no evidence for well No. SW 23 but it could be sulphate.

There were two areas with high levels of sodium at the site; one area includes wells No. SW 5, SW 14 - SW 18 and well water pump house; and the other area includes wells No. SW 13, TW 2 - TW 4, TW 6, TW 8 - TW 11, TW 104 - TW 114, BH 5, BH 7, BH 9 and BH 10; and the North and South streams. Sodium level increases with the depth of the wells (total depth in the range from 11 - > 20 metres) [see Table 4.8.]. In addition, The high levels of sodium occurred in wells with lower water levels in the range from 3.2 - 5.0 metres below ground surface (see Table 4.7.). Sodium contamination was widespread at the site.

Of the all wells, there were no great seasonal variations of sodium level in about two thirds of the wells and the other third of the wells had variable levels of sodium except wells No. SW 102 and TW 4 had very variable levels of sodium.

4.3.1.7. <u>POTASSIUM</u>

The seasonal variations of potassium in the groundwater monitoring wells are given in Table 4.17. The chemical composition of the landfill groundwater monitoring wells (Spring, 1997) is shown in Table 4.11. As can be seen, the natural background level of potassium was < 10 mg K /l at the site. Two thirds of the wells were contaminated with potassium particularly wells No. SW 5, SW 10, SW 13, SW 14, SW 16, Misk Mine, TW 11, TW 104, TW 105, TW 106, TW 109, TW 112, TW 113, BH 7, BH 9 and BH 10. Well No. SW 15 was extremely contaminated. The North and South streams were contaminated.

There were two areas with high levels of potassium at the site. The first area includes wells No. SW 5 and SW 14 - SW 16; and the second area includes well No. SW 13, TW 11, TW 104 - TW 106, TW 109, TW 112, TW 113, BH 7, BH 9 and BH 10, and the North and South streams. Potassium increases with the depth of the wells

(total depth in the range from 10 - 19 metres) [see Table 4.8.]. Potassium contamination was widespread at the site.

There were no great seasonal variations of potassium level in the majority of the wells except wells No. SW 5, SW 13, SW 15 and well water pump house had variable levels of potassium and well No. Misk Mine had a very variable level of potassium.

4.3.1.8. CALCIUM

The seasonal variations of calcium in the groundwater monitoring wells are demonstrated in Table 4.18. The chemical composition of the landfill groundwater monitoring wells (Spring, 1997) is shown in Table 4.11. It was observed that the natural background level of calcium was < 50 mg Ca /l at the site. Two thirds of the wells were contaminated with calcium. Wells No. SW 15, SW 16, TW 5 and TW 8 are extremely contaminated. The North and South streams were contaminated.

There were three areas with high levels of calcium in the site; the first area includes wells No. SW 5, SW 8B, SW 14 - SW 19 and well water pump house; and the second area includes wells No. SW 2, SW 13, TW 3 TW 4 - TW 11, TW 104 - TW 114, BH 5, BH 7, BH 9 and BH 10; and the North and South streams. The third area includes wells No. SW 104 and SW 107. Calcium increases with the depth of the wells (total depth in the range from 13 - 20 metres) [see Table 4.8.]. Calcium contamination was widespread at the site.

Of all of the wells, there were no great seasonal variations of calcium level in more than half of the wells but about quarter of the wells had variable levels of calcium and six wells (wells No. SW 2, SW 5, SW 8B, SW 15, TW 3 and Misk Mine) had very variable levels of calcium.

Table 4.15. : Seasonal variations of chloride (mg /l) in the groundwater monitoring wells.

Well No.	Autumn (1996)	Spring (1997)	Summer (1997)
SW 1	56	46.6	----
SW 2	40.4	30.4	51
SW 3	46.5	54	70
SW 4	98	81	95
SW 5	3332	3637	3547
SW 6	----	22.4	----
SW 7	85	91	----
SW 8B	----	63	47.2
SW 9	70	84	76
SW 10	----	31.3	35.3
SW 11	21.8	23.5	26.7
SW 12	19.8	28.0	----
SW 13	3570	2539	----
SW 14	----	144	----
SW 15	450	458	----
SW 16	----	90	----
SW 17	102	127	164
SW 18	----	37.1	----
SW 19	33.9	40.4	----
SW 20	87	75	----
SW 21	37.1	----	----
SW 22	42.6	38.8	----
SW 23	40.6	40.8	----
SW 101	38.0	41.8	40.5
SW 102	----	37.3	38.0
SW 103 (upper)	17.8	39.1	35.2
SW 103 (lower)	125	123	179
SW 104	24.7	21.1	27.5
SW 107	----	15.1	23.8
TW 1	----	87	79
TW 2	----	213	261
TW 3	----	1527	1713
TW 4	2902	1779	----
Misk Mine	492	426	----
Well water pump house	205	208	----

---- No sample collected.

Table 4.16. : Seasonal variations of sodium (mg /l) in the groundwater monitoring wells.

Well No.	Autumn (1996)	Spring (1997)	Summer (1997)
SW 1	27.0	30.0	----
SW 2	59	53	47.3
SW 3	31.8	63	37.5
SW 4	70	53	63
SW 5	2170	2225	2175
SW 6	----	35.0	----
SW 7	67	64	----
SW 8B	----	50	44.5
SW 9	35.0	43.8	42.5
SW 10	----	32.5	41.0
SW 11	32.0	25.6	23.8
SW 12	12.0	21.3	----
SW 13	2450	2100	----
SW 14	----	413	----
SW 15	1520	1625	----
SW 16	----	5600	----
SW 17	92	118	140
SW 18	----	1600	----
SW 19	53	46.3	----
SW 20	137	115	----
SW 21	31.0	----	----
SW 22	40.0	28.1	----
SW 23	455	350	----
SW 101	25.0	25.6	23.8
SW 102	----	63	21.8
SW 103 (upper)	22.0	28.8	16.3
SW 103 (lower)	220	256	274
SW 104	22.0	18.8	20.0
SW 107	----	22.5	36.0
TW 1	----	51	46.0
TW 2	----	90	107
TW 3	----	975	675
TW 4	1850	1175	----
Misk Mine	368	406	----
Well water pump house	160	213	----

---- No sample collected.

Table 4.17. : Seasonal variations of potassium (mg /l) in the groundwater monitoring wells.

Well No.	Autumn (1996)	Spring (1997)	Summer (1997)
SW 1	4.2	3.8	----
SW 2	14.0	20.0	27.5
SW 3	3.2	3.8	4.1
SW 4	3.8	3.3	3.9
SW 5	170	175	144
SW 6	----	4.1	----
SW 7	15.0	17.5	----
SW 8B	----	2.5	6.0
SW 9	4.0	3.4	3.2
SW 10	----	80	82
SW 11	6.5	2.5	4.2
SW 12	1.9	2.5	----
SW 13	74	58	----
SW 14	----	132	----
SW 15	788	950	----
SW 16	----	100	----
SW 17	18.8	10.0	20.5
SW 18	----	21.0	----
SW 19	9.5	5.0	----
SW 20	4.5	4.3	----
SW 21	5.0	----	----
SW 22	30.0	22.5	----
SW 23	45.0	32.5	----
SW 101	15.0	10.0	12.5
SW 102	----	3.2	3.7
SW 103 (upper)	2.9	3.0	3.6
SW 103 (lower)	10.0	7.5	10.5
SW 104	3.1	3.2	3.4
SW 107	----	2.3	2.5
TW 1	----	4.5	4.2
TW 2	----	17.5	19.3
TW 3	----	32.5	36.3
TW 4	60	63	----
Misk Mine	18.1	105	----
Well water pump house	12.0	27.5	----

---- No sample collected.

Table 4.18. : Seasonal variations of calcium (mg /l) in the groundwater monitoring wells.

Well No.	Autumn (1996)	Spring (1997)	Summer (1997)
SW 1	59	69	----
SW 2	153	3.1	118
SW 3	15.8	38.0	25.0
SW 4	31.3	29.0	36.0
SW 5	51	102	73
SW 6	----	29.5	----
SW 7	49.8	48.1	----
SW 8B	----	66	118
SW 9	94	60	64
SW 10	----	49.0	46.0
SW 11	34.9	43.0	40.0
SW 12	16.8	22.0	----
SW 13	81	66	----
SW 14	----	390	----
SW 15	1950	1160	----
SW 16	----	470	----
SW 17	89	99	113
SW 18	----	212	----
SW 19	118	112	----
SW 20	26.8	30.0	----
SW 21	73	----	----
SW 22	54	42.1	----
SW 23	125	99	----
SW 101	10.0	9.0	7.0
SW 102	----	30.0	29.0
SW 103 (upper)	67	70	61
SW 103 (lower)	21.8	22.0	23.5
SW 104	87	91	94
SW 107	----	98	106
TW 1	----	20.7	22.0
TW 2	----	26.0	11.0
TW 3	----	78	130
TW 4	149	115	----
Misk Mine	126	35.0	----
Well water pump house	76	70	----

---- No sample collected.

4.3.1.9. MAGNESIUM

The seasonal variations of magnesium in the groundwater wells are listed in Table 4.19. The chemical composition of the landfill groundwater monitoring wells (Spring, 1997) is shown in Table 4.11. As can be seen, the natural background level of magnesium was < 30 mg Mg /l at the site. More than one third of the wells was contaminated with magnesium. Wells No. SW 15, SW 16, TW 8, BH 7, BH 9 and BH 10 were extremely contaminated. The North and South streams were also extremely contaminated.

There were two areas with high levels of magnesium in the site; the first area includes wells No. SW 14 - SW 16, SW 18 and SW 19; and the second area includes wells No. SW 2, SW 13, TW 3 - TW 5, TW 8, TW 11, TW 104 - TW 106, TW 109, TW 110, TW 112 - TW 114, BH 5, BH 7, BH 9 and BH 10, and the North and South streams. Magnesium level increases with the depth of the wells (total depth in the range from 13 - 19 metres) [see Table 4.8.]. Magnesium contamination was widespread at the site.

Of the all wells, there were no great seasonal variations in about two thirds of the wells and there is variability in the level of magnesium in the other third of the wells except wells No. SW 15, SW 19 and TW 4 had very variable levels of magnesium.

4.3.1.10. IRON

The seasonal variations of iron in the groundwater monitoring wells are presented in Table 4.20. The chemical composition of the landfill groundwater monitoring wells (Spring, 1997) is shown in Table 4.11. It was noticed that the natural background level of iron was < 1.0 mg Fe /l at the site. Two thirds of the wells were contaminated with iron. Wells No. TW 5 and TW 106 were extremely contaminated. The North stream was not contaminated but South stream was contaminated.

The high levels of iron are likely to be due to the acidic pH as evidenced by the high levels of iron and acidic pH in wells No. SW 2, SW 14, SW 16, TW 5, TW 104 - TW 108, TW 112 and TW 114; and the South stream.

There were three areas with high levels of iron at the site; the first area includes wells No. SW 2, TW 4 - TW 6, TW 8, TW 11, TW 104 - TW 108, TW 111,

TW 112 and TW 114, and the South stream; the second area includes well No. SW 11; and the third area includes wells No. SW 14 and SW 16. Iron contamination was widespread at the site.

There were no great seasonal variations of iron level in the majority of the wells except wells No. TW 1 and SW 8B had variable levels of iron and wells No. SW 2, SW 11, SW 103 (lower) and SW 107 had very variable levels of iron.

4.3.1.11. ELECTRICAL CONDUCTIVITY

The seasonal variations of the electrical conductivity in the groundwater monitoring wells are listed in Table 4.21. The chemical composition of the landfill groundwater monitoring wells (Spring, 1997) is shown in Table 4.11. As can be seen, the natural background level of electrical conductivity was < 1.0 ms /cm at the site. More than one third of the wells had high electrical conductivity particularly wells No. SW 2, SW 5, SW 13, SW 18, TW 4, TW 108 and BH 10. Wells No. SW 14, SW 15, SW 16, BH 7 and BH 9 had very high electrical conductivity. The South stream had high electrical conductivity and the North stream had very high electrical conductivity.

The high electrical conductivity in wells No. SW 5, SW 14, SW 15, SW 16 and SW 18 is due to high levels of anions such as chloride, ammonium and nitrate, and high levels of cations such as sodium, potassium, calcium, magnesium and iron. The high electrical conductivity in wells No. TW 4, SW 13, SW 2, BH 7, BH 9 and BH 10; and the North and South streams is due to high levels of anions such as chloride, and high levels of cations such as potassium, sodium, calcium, magnesium and iron (in case of wells No. TW 4, SW 13 and SW 2; and the South stream). The electrical conductivity increases in wells with higher water levels in the range from 1.1 - 3.8 metres below ground surface (see Table 4.8.) and this could be attributed to the salinity of the drainage water. The high electrical conductivity of well water No. TW 108 is due to high levels of anions such as chloride and high levels of cations such as sodium, calcium and iron.

Although wells No. SW 14, SW 15 and SW 16 are close to each other; they are not uniformly high in some contaminants such as ammonium, nitrate, chloride, potassium, sodium, calcium, magnesium and iron. Well No. SW 15 was the most badly contaminated well on the site.

There are two areas with high electrical conductivity in the site; the first area includes wells No. SW 5, SW 18 and SW 14 - SW 16; and the second area includes wells No. SW 2, SW 13, TW 4, TW 108, BH 7, BH 9 and BH 10, and the North and South streams.

The high levels of chloride and sodium could be due to saline water intrusion from the sea (Firth of Clyde) as in wells No. SW 20, SW 13, TW 2 and TW 3 since they were close to the coast. In addition, well No. SW 13 is close to the tidal River Irvine. However, wells No. SW 1 and SW 4 had very low electrical conductivity, and moderate levels of chloride and sodium although they were close to the coast of the sea (Firth of Clyde) suggesting it is not saline water intrusion from the sea. Therefore, these high levels of chloride and sodium, and high electrical conductivity could be due to chloride or sodium contamination and this is evidenced by the high levels of chloride and sodium in wells No. TW 8, TW 10, TW 11, TW 104 - TW 114 and Misk Mine; and high electrical conductivity in wells No. SW 2 and TW 108 although they were very close to River Garnock. Moreover; wells No. BH 7, BH 9 and BH 10 as well as the North and South streams were between the high water line (tide) and the bank of River Garnock; but they had very high levels of chloride and sodium, and high electrical conductivity.

There were no great seasonal variations of the electrical conductivity in the majority of the wells except wells No. SW 2 and TW 4 had variable electrical conductivity.

Table 4.19. : Seasonal variations of magnesium (mg /l) in the groundwater monitoring wells.

Well No.	Autumn (1996)	Spring (1997)	Summer (1997)
SW 1	7.0	8.2	----
SW 2	65	79	39.3
SW 3	4.3	17.1	8.5
SW 4	15.0	13.0	17.2
SW 5	8.3	13.4	14.7
SW 6	----	5.1	----
SW 7	28.0	29.5	----
SW 8B	----	18.7	28.6
SW 9	18.0	13.4	13.6
SW 10	----	25.6	25.0
SW 11	4.3	1.7	2.5
SW 12	3.3	3.9	----
SW 13	235	203	----
SW 14	----	175	----
SW 15	2960	2425	----
SW 16	----	2360	----
SW 17	33.0	35.7	45.3
SW 18	----	381	----
SW 19	135	208	----
SW 20	7.3	10.9	----
SW 21	8.0	----	----
SW 22	12.0	10.2	----
SW 23	140	110	----
SW 101	7.3	6.6	5.2
SW 102	----	8.6	9.5
SW 103 (upper)	4.3	5.5	3.9
SW 103 (lower)	7.3	9.1	7.7
SW 104	2.5	3.0	3.5
SW 107	----	3.7	4.7
TW 1	----	19.3	18.3
TW 2	----	28.1	28.6
TW 3	----	141	113
TW 4	220	323	----
Misk Mine	78	92	----
Well water pump house	41.0	48.0	----

---- No sample collected.

Table 4.20. : Seasonal variations of iron (mg /l) in the groundwater monitoring wells.

Well No.	Spring (1997)	Summer (1997)
SW 1	< 0.05	----
SW 2	301	182
SW 3	8.0	10.5
SW 4	0.1	0.2
SW 5	0.1	3.9
SW 6	48.0	----
SW 7	15.0	----
SW 8B	21.0	17.6
SW 9	9.5	9.0
SW 10	39.0	37.0
SW 11	9.5	97
SW 12	0.3	----
SW 13	0.2	----
SW 14	391	----
SW 15	4.3	----
SW 16	386	----
SW 17	< 0.05	< 0.05
SW 18	54.0	----
SW 19	15.0	----
SW 20	0.1	----
SW 21	----	----
SW 22	21.0	----
SW 23	< 0.05	----
SW 101	0.5	0.2
SW 102	36.5	32.5
SW 103 (upper)	< 0.05	0.1
SW 103 (lower)	15.0	0.6
SW 104	2.4	0.6
SW 107	28.5	53
TW 1	8.0	4.1
TW 2	0.2	0.5
TW 3	< 0.05	< 0.05
TW 4	61	----
Misk Mine	3.9	----
Well water pump house	< 0.05	----

---- No sample collected.

Table 4.21. : Seasonal variations of the electrical conductivity [EC (ms /cm)] in the groundwater monitoring wells.

Well No.	Autumn (1996)	Spring (1997)	Summer (1997)
SW 1	0.4	0.5	----
SW 2	7.3	14.8	10.5
SW 3	0.3	0.6	0.3
SW 4	0.5	0.5	0.6
SW 5	8.5	9.9	9.8
SW 6	----	2.9	----
SW 7	0.7	0.8	----
SW 8B	----	0.8	1.1
SW 9	0.7	0.6	0.6
SW 10	----	0.8	0.8
SW 11	0.8	0.6	0.5
SW 12	0.2	0.2	----
SW 13	9.9	7.9	----
SW 14	----	75	----
SW 15	79	83	----
SW 16	----	52	----
SW 17	1.0	1.1	1.3
SW 18	----	11.0	----
SW 19	1.4	1.3	----
SW 20	0.7	0.7	----
SW 21	0.5	----	----
SW 22	0.5	0.5	----
SW 23	2.7	2.2	----
SW 101	0.3	0.3	0.2
SW 102	----	0.3	0.3
SW 103 (upper)	0.4	0.4	0.4
SW 103 (lower)	1.1	1.2	1.2
SW 104	0.5	0.5	0.5
SW 107	----	0.5	0.7
TW 1	----	0.5	0.5
TW 2	----	0.9	1.0
TW 3	----	4.7	4.5
TW 4	7.9	5.8	----
Misk Mine	2.4	2.3	----
Well water pump house	1.3	1.4	----

---- No sample collected.

4.3.2. CHARACTERISATION OF THE SOILS

Three of the four areas, IOP, H-Acid and Safety Fuse, have imported topsoil overlying building rubble, stony rubble, asphalt rubble, concrete, brick and ash. The other area, the Nylon area, is sandy with some development of a natural topsoil.

There are variations in the vegetation and this could be due to toxicity or lack of nutrients.

4.3.2.1. CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE SOILS

4.3.2.1.1. INTERMEDIATE OXIDATION PLANT (IOP) AREA

The chemical and physical characteristics of the soil in the IOP area are presented in Table 4.22. The results are the mean of two replicates. Additional analysis (electrical conductivity, pH, moisture content, moisture content at - 0.5 bar, ammonium-N, nitrate-N, nitrite-N, potassium, phosphorus, magnesium, cation exchange capacity and mechanical analysis) was carried out on soil samples selected for detailed study. The results are presented in section 6.3.1. As can be seen from Table 4.22., the following could be drawn :

The pH is slightly acidic and variable in the topsoils. The pH is variable in the subsoils. The pH of sample No. 1c is high (8.3) as this material has building rubble and would contain significant levels of mortar. The electrical conductivity is low and variable in the topsoils and subsoils. Sample No. 1a has a slightly higher electrical conductivity and this may be due to surface contamination. The organic matter content is low and variable in the topsoils and subsoils. Samples No. 2b, 2c and 2d had high organic matter content since they were contaminated with ash material. Ammonium, nitrate, phosphorus and potassium levels are very low and variable in the topsoils and subsoils. Sample No. 1c had high level of potassium. In the field, vegetation of sites No. 1, 2 and 3 was related to the growth of white clover. These low levels of nutrients (N, P and K) are coincide with the poor vegetation of sites No. 4 and 5.

4.3.2.1.2. H-ACID AREA

The chemical and physical characteristics of the soil in the H-Acid area are presented in Table 4.22.

The pH is slightly acidic and approximately similar in the topsoils. The pH of subsoil (sample No. 9b) is high (7.8) as this material has building rubble and contains significant levels of mortar. The electrical conductivity is very low in the topsoils and subsoil. The organic matter content is high and variable in the topsoils, and is very low in the subsoil. Ammonium and nitrate levels are low and variable in the topsoils and subsoil. Phosphorus and potassium levels are slightly higher than those of the IOP area and variable in the topsoils. Phosphorus and potassium levels are low in the subsoil.

The topsoil of the H-Acid area is different from those of the IOP and Safety Fuse area. In addition, in the H-Acid topsoils, there is more available nitrogen, phosphorus and potassium than those of the IOP, Safety Fuse and Nylon areas; and this coincide with better vegetation [white clover mixed with grass (full coverage, about 50 % : 50 % mix)].

4.3.2.1.3. SAFETY FUSE AREA

The chemical and physical characteristics of the soil in the Safety Fuse area are presented in Table 4.22.

The pH is slightly acidic and variable in the topsoils while the pH is neutral and approximately similar in the subsoils. The electrical conductivity is low and variable in the topsoils, but it is very low and similar in the subsoils. The electrical conductivity of samples No. 10a and 12a is slightly higher suggesting surface contamination. The organic matter content is very low and variable in the topsoils and subsoils. Ammonium level is very low and approximately similar in the topsoils, while it is very low and variable in the subsoils. Nitrate level is very low and variable in the topsoils and subsoils. In the field, the vegetation of sites No. 11 and 12 was related to the growth of white clover. Phosphorus level is low and variable in the topsoils, but it is very low and variable in the subsoils. Potassium level is low and variable in the topsoils and subsoils.

The Safety Fuse area was topsoiled with the same topsoil as that of the Intermediate Oxidation Plant (IOP) area , however, it tends to have different properties.

These low levels of nutrients (N, P and K) are coincide with the poor vegetation of sites No. 10 and 13.

4.3.2.1.4. NYLON AREA

The chemical and physical characteristics of the soil in the Nylon area are presented in Table 4.22.

The pH is slightly higher but variable in the topsoils, and is high in the subsoil. The electrical conductivity is very low in the topsoils and subsoil. The organic matter content is very low in the topsoils and subsoil, while it is variable in the topsoils. Ammonium and nitrate levels are very low in the topsoils and subsoil, and are variable in the topsoils. Phosphorus and potassium levels are low and variable in the topsoils and subsoil, however, the topsoil of site No. 15 is high in the extractable phosphate.

There is reasonable cover of legumes (trefoil and gorse). However, these low levels of N, P and K are due to that the Nylon area had natural soil which needs time for development.

Table 4.22. : Chemical and physical characteristics of the soil in the IOP, H-Acid, Safety Fuse and Nylon areas.

Sample No.	pH	EC * (ms /cm)	Organic matter (%)	NH_4-N (mg/kg)	NO_3-N (mg/kg)	P (mg/kg)	K (mg/kg)
IOP AREA :							
1 a	5.6	1.6	4.3	1.0	< 0.1	10.6	56
1 b	5.1	1.1	3.8	0.8	< 0.1	13.1	31.7
1 c	8.3	0.4	5.2	0.6	0.6	4.6	262
2 a	6.6	0.2	3.5	1.0	< 0.1	9.9	32.3
2 b	6.3	0.1	10.3	0.8	0.4	2.9	32.7
2 c	6.5	0.1	14.6	1.0	0.6	9.1	19.5
2 d	7.0	0.1	9.3	0.8	1.6	3.2	34.2
3 a	5.6	0.1	4.9	1.4	< 0.1	13.9	33.6
4 a	5.3	0.1	4.4	1.0	< 0.1	17.1	42.1
5 a	4.1	0.2	2.3	2.4	0.8	17.1	7.8
H-ACID AREA :							
6 a	5.8	0.1	11.6	5.9	5.3	42.9	45.3
7 a	5.9	0.1	11.1	15.8	12.7	26.5	106
8 a	5.4	0.1	7.0	3.9	11.9	85	150
9 a	5.9	0.1	18.7	7.9	2.0	16.4	71
9 b	7.8	0.2	1.7	5.0	1.4	3.8	28.2

* EC = Electrical conductivity.

Table 4.22. : Continued.

Sample No.	pH	EC * (ms /cm)	Organic matter (%)	NH_4-N (mg/kg)	NO_3-N (mg/kg)	P (mg/kg)	K (mg/kg)
SAFETY FUSE AREA :							
10 a	4.9	2.0	2.9	0.8	< 0.1	7.1	34.2
10 b	7.0	0.1	1.5	0.4	0.2	4.6	33.9
10 c	7.5	0.1	0.9	0.2	< 0.1	2.8	37.1
11 a	6.0	0.2	2.7	0.8	< 0.1	7.7	21.3
12 a	7.0	1.6	3.4	1.0	< 0.1	10.5	37.9
13 a	4.4	1.2	2.9	1.0	0.2	8.5	20.4
13 b	7.3	0.1	0.6	0.8	0.2	2.2	32.0
NYLON AREA :							
14 a	7.7	0.1	1.3	1.2	0.8	10.0	19.3
15 a	6.9	0.1	2.0	2.0	2.6	66	52
16 a	7.4	0.1	3.2	2.2	1.6	4.9	17.7
17 a	7.6	0.1	2.3	2.4	1.4	3.2	18.6
17 b	8.4	0.1	0.6	0.6	0.6	1.4	6.8

* EC = Electrical conductivity.

4.3.2.2. ASSESSMENT OF THE TOXICITY AND THE HAZARDS OF THE HEAVY METALS

The heavy metals content of the soil in the Intermediate Oxidation Plant (IOP), H-Acid, Safety Fuse and Nylon areas is given in Tables 4.23, 4.24. and 4.25. The results are the mean of two replicates.

As can be seen from Tables 4.23., 4.24. and 4.25., cadmium (wavelength = 226.502 nm) and chromium (wavelength = 283.563 nm) measured by the Inductively Coupled Plasma (ICP) are higher than by the Atomic Absorption Spectrophotometer (AAS). This is likely to be due to spectral overlap caused by iron. As cadmium and chromium were analysed in the same soil digest by the two techniques (ICP and AAS), the error is due to the instrumental analysis. These two choices of wavelengths (Cd and Cr) are the standard wavelengths selected by analysts at Ardeer. Thus, the AAS results were used.

As can be seen from Table 4.23., in general, the levels of toxic metals are low in the soils. The slightly elevated levels are to be associated with ash and rubble materials in the IOP area, and with the rubble material in the H-Acid area.

As can be seen from Tables 4.24 and 4.25., samples No. 1c, 2b, 2c, 2d and 9a had slightly higher levels of barium than is typical of the site. The high levels of barium in these samples could be due to building rubble, concrete, brick and ash material.

Relative to the ICRCL Guidance Note 59 /83 (1987) shown in Table 4.26., some samples exceeded the threshold value such as samples No. 2b and 17a (copper), and samples No. 2d, 9a and 9b (nickel). Certain samples were on the borderline of the threshold value such as samples No. 1c and 17a (zinc), and sample No. 2b (nickel).

4.3.2.3. OVERALL ASSESSMENT OF THE AREAS

The purpose of this study was to select sites for soil sampling for nitrification and pot experiments.

It was concluded that the heavy metals are not expected to cause problems with growth of vegetation or microbial nutrients turnover. In some cases, the pH is borderline for nitrification, although none of the sites appear likely to cause problems for vegetation growth. Overall, the variations in vegetation cover appear to be due to

differences in macronutrient availability rather than any toxicity. Many soils are very sandy and prone to leaching and low in organic matter content and available nitrogen.

Table 4.23. : Heavy metals content of the soil in the IOP, H-Acid, Safety Fuse and Nylon areas*.

Sample No.	Cu (mg /kg)	Cd (mg /kg)	Cr (mg /kg)	Zn (mg /kg)	Ni (mg /kg)	Pb (mg /kg)
IOP AREA :						
1 a	32.4	0.6	25.9	133	44.5	51
1 b	15.9	0.4	22.9	75	29.4	31.4
1 c	84	1.8	22.7	293	46.6	358
2 a	16.4	< 0.4	21.7	81	26.7	38.3
2 b	165	0.8	33.3	255	69	339
2 c	37.9	< 0.4	14.5	48.0	48.4	41.3
2 d	81	0.6	21.9	135	73	165
3 a	20.8	1.0	31.6	124	38.8	40.8
4 a	17.1	0.8	27.4	79	30.0	96
5 a	7.7	< 0.4	21.5	32.0	19.2	11.5
H-ACID AREA :						
6 a	34.2	0.8	39.2	175	41.6	72
7 a	31.9	1.0	39.6	166	50	66
8 a	15.5	0.4	40.9	66	24.0	30.0
9 a	48.2	1.6	82	196	92	80
9 b	11.7	< 0.4	12.4	54	107	26.5

* The heavy metals were determined by the Atomic Absorption Spectrophotometer.

Table 4.23. : Continued.

Sample No.	Cu (mg /kg)	Cd (mg /kg)	Cr (mg /kg)	Zn (mg /kg)	Ni (mg /kg)	Pb (mg /kg)
SAFETY FUSE AREA :						
10 a	11.3	< 0.4	24.1	64	24.1	35.4
10 b	4.2	< 0.4	17.2	31.6	17.6	11.2
10 c	2.0	< 0.4	21.0	33.6	23.6	1.0
11 a	13.4	< 0.4	22.3	56	26.3	38.1
12 a	22.1	0.4	22.7	89	31.8	62
13 a	7.9	0.4	21.7	34.0	20.0	26.1
13 b	1.6	< 0.4	15.0	18.0	14.0	< 1.0
NYLON AREA :						
14 a	41.6	< 0.4	19.6	110	14.8	14.6
15 a	6.3	< 0.4	20.2	123	17.2	9.5
16 a	18.4	< 0.4	27.3	115	13.8	15.6
17 a	181	< 0.4	23.9	298	20.5	35.6
17 b	2.8	< 0.4	12.4	18.8	10.2	10.4

Table 4.24. : Metals content of the soil in the IOP and H-Acid areas*.

Sample No.	Cd (mg /kg)	Cr (mg /kg)	Zn (mg /kg)	Ni (mg /kg)	Pb (mg /kg)
IOP AREA :					
1 a	2.8	54	131	39.7	38.3
1 b	2.9	54	76	29.6	23.3
1 c	4.9	55	300	40.7	380
2 a	2.2	47.2	83	25.9	38.5
2 b	5.1	76	227	57	314
2 c	2.4	28.8	41.9	49.6	40.9
2 d	5.9	45.8	109	59	126
3 a	4.5	61	133	40.0	46.9
4 a	3.3	60	83	31.2	98
5 a	1.6	38.5	33.2	19.8	7.9
H-ACID AREA :					
6 a	7.8	104	174	38.0	42.8
7 a	7.9	107	175	48.8	38.1
8 a	3.5	75	66	24.8	14.1
9 a	8.1	160	205	85	18.5
9 b	1.2	21.9	57	108	22.7

* The metals were determined by the Inductively Coupled Plasma (ICP).

Table 4.24. : Continued.

Sample No.	B (mg /kg)	Ba (mg /kg)	Mn (mg /kg)	Mg (mg /kg)	K (mg /kg)
IOP AREA :					
1 a	34.0	100	345	3916	890
1 b	28.6	56	256	3704	598
1 c	47.8	244	322	3094	1097
2 a	25.9	60	288	3641	536
2 b	55	190	550	2693	865
2 c	30.0	172	130	824	337
2 d	46.4	226	314	2629	546
3 a	43.7	97	444	3711	620
4 a	30.8	61	382	3601	598
5 a	23.1	35.8	122	3145	482
H-ACID AREA :					
6 a	66	80	1268	3997	1007
7 a	74	81	1210	4164	1092
8 a	34.5	50.2	353	2468	848
9 a	77	245	381	4515	1437
9 b	17.0	113	80	1055	374

Table 4.25. : Metals content of the soil in the Safety Fuse and Nylon areas*.

Sample No.	Cd (mg /kg)	Cr (mg /kg)	Zn (mg /kg)	Ni (mg /kg)	Pb (mg /kg)
SAFETY FUSE AREA :					
10 a	1.8	42.1	59	30.8	28.1
10 b	1.2	30.0	29.8	18.0	8.2
10 c	1.4	35.6	33.4	25.0	< 0.0
11 a	1.8	41.1	59	27.1	30.2
12 a	2.4	45.6	100	32.4	54
13 a	1.4	36.8	37.7	21.1	19.0
13 b	1.0	25.6	21.2	14.8	< 0.0
NYLON AREA :					
14 a	1.0	31.6	126	15.8	14.8
15 a	1.4	34.0	139	17.2	2.0
16 a	1.2	43.3	144	14.8	11.1
17 a	1.2	37.7	314	19.6	30.8
17 b	1.0	23.0	23.2	10.4	7.0

* The metals were determined by the Inductively Coupled Plasma (ICP).

Table 4.25. : Continued.

Sample No.	B (mg /kg)	Ba (mg /kg)	Mn (mg /kg)	Mg (mg /kg)	K (mg /kg)
SAFETY FUSE AREA :					
10 a	20.2	49.0	210	3441	566
10 b	21.4	14.0	89	2507	396
10 c	18.6	13.4	111	3407	475
11 a	18.0	53	205	3927	546
12 a	36.2	84	278	4501	705
13 a	15.6	38.1	129	3157	514
13 b	16.0	10.6	130	2089	382
NYLON AREA :					
14 a	33.4	98	174	1596	338
15 a	34.0	29.4	183	2469	456
16 a	25.5	68	347	1938	380
17 a	25.7	77	209	2030	381
17 b	18.6	16.0	113	1519	304

Table 4.26. : ICRCL Guidance Note 59 /83 (1987) for the assessment of contaminated land.

Contaminants	Planned uses	Trigger concentrations (mg /kg air dried soil)	
		Threshold	Action
GROUP (A) [1] :			
Cadmium	Parks, playing fields and open space.	15	*
Chromium (total)	Parks, playing fields and open space.	1000	*
Lead	Parks, playing fields and open space.	2000	*
GROUP (B) [2] :			
Copper (3)	Any uses where plants to be grown (4 and 5).	130	*
Nickel (3)	Any uses where plants to be grown (4 and 5).	70	*
Zinc (3)	Any uses where plants to be grown (4 and 5).	300	*

Notes :

* Action concentrations will be specified in the next edition of ICRCL 59 /83.

1 = Group (A) is contaminants which may pose hazards to health.

2 = Group (B) is contaminants which are phytotoxic but not normally hazardous to health.

3 = The phytotoxic effects of copper, nickel and zinc may be additive. The trigger values given here are those applicable to the "worst-case" : phytotoxic effects may occur at these concentrations in acid, sandy soils. In neutral or alkaline soils, phytotoxic effects are unlikely at these concentrations.

4 = The soil pH value is assumed to be about 6.5 and should be maintained at this value. If the pH falls, the toxic effects and the uptake of these elements will be increased.

5 = Grass is more resistant to phytotoxic effects than are most other plants and its growth may not be adversely affected at these concentrations.

CHAPTER 5

NITRIFICATION IN SOIL TREATED WITH CONTAMINATED GROUNDWATER

5.1. INTRODUCTION

The interest in nitrification in this chapter is because it is a useful indicator of soil health and the activity of micro-organisms in soil because it is a vulnerable part of the nitrogen cycle and the micro-organisms involved are sensitive to inhibitors. In addition, the rate of nitrification is important as if the soil nitrify ammonium slowly, ammonium is more likely to remain in the topsoil allowing the plant to make use of it before it is leached as nitrate. Nitrification is very sensitive and can be inhibited by different chemical compounds such as heavy metals and organic compounds. There are several environmental factors which have an affect on nitrification such as soil water content, oxygen supply, pH, temperature and the presence or absence of the plant.

Okpokwasili and Odokuma (1996) investigated the effect of drilling chemicals on nitrate utilisation and logarithmic rate of growth of *Nitrobacter* using varying concentrations of the chemicals. They noted that the drilling chemicals are toxic to *Nitrobacter* through inhibition of nitrite oxidation to nitrate and cell mortality. The order of toxicity is Carbotrol A9 > Carbomul Sea > Carbotec Sea > Carbovis > Carbotec HW > Huile-clean > Chaux (lime). Nitrite utilisation by *Nitrobacter* increased with increase in exposure time to the chemicals and decreased with increase in concentration of the chemicals. However, some concentrations of drilling chemicals stimulated the growth rate of *Nitrobacter* as exposure time increased.

Sinkjaer et al. (1996) reported that the scrubber water at the Lynetten wastewater treatment plants inhibited nitrification but it was not possible to identify with any certainty the specific substances or compounds producing this effect. However, it was possible to rule out heavy metals and several organic inhibitors known from literature. It was found that the inhibitory effect was reduced, if the flue gas was incinerated at temperatures above 850 °C. Furthermore, it has been established that biological treatment of the scrubber water could reduce the inhibition considerably.

The moisture content of soil has an effect on nitrification. In the current nitrification study, the moisture content of the soils used was -0.5 bar moisture potential which is considered to be an optimum moisture content for the activity of the micro-organisms.

Flowers and O'Callaghan (1983) indicated that nitrification rate increased with increasing moisture content up to the highest level tested, soil water potential -8.0 kPa, corresponding to approximately 60 % of water holding capacity in both soils. Despite the lowest moisture content (soil water potential -1.5 MPa) and temperature (5 °C) tested, measurable nitrification was found in both soils.

Grundmann et al. (1995) concluded that nitrification was generally maximum when the intra-aggregate pore spaces were saturated with water, but with no water in the inter-aggregate pore spaces.

Alexander (1977) pointed out that among the ecological influences that affect the rate of ammonium oxidation is acidity. In acid environments, nitrification proceeds slowly even in the presence of an adequate supply of substrate, and the responsible species are rare or totally absent. Typically, the rate falls off markedly below pH 6.0 and becomes negligible below 5.0 (Dancer et al., 1973), yet, nitrate may occasionally be present in fields down to 4.0 and sometimes lower. Some soils nitrify at 4.5. This may be due to acid-adapted strains but is often attributed to heterotrophic bacteria. The acidity affects not only the transformation itself but also the microbial numbers, neutral to alkaline soils having the largest populations. The pH values for growth of these bacteria depend to some extent on the locality from which they originated. Strains derived from acid soils are frequently more tolerant of high hydrogen ion concentration than those from areas of alkaline pH, and the optimum pH for individual isolates may vary from as low as 6.6 to as high as 8.0 or sometimes higher.

Flowers and O'Callaghan (1983) reported that nitrification of 100 μg NH_4-N /g soil resulted in a fall of 0.5 pH units in both soils used but no decrease in the rate. Three samples of Dunkeswick series soil showed a decrease in nitrification rate associated with a decrease in initial pH from 6.2 to 5.8. This difference may have been due to lower populations of nitrifying organisms or lower rate in the more acid samples but again there was no effect of the fall in pH during the incubation.

Shah (1988) showed that the lack of nitrification of added ammonium sulphate or urea nitrogen in the coal mine soil may have been due to its low pH (3.8), because of the sensitivity of nitrifying bacteria to pH. Nitrification of added ammonium-N in the form of chicken manure could be attributed to the increase in pH of the coal mine soil due to manure addition, which favoured the activity of nitrifying bacteria in this strongly acid soil.

The temperature has an effect on nitrification as nitrification increases with increase in temperature. The current nitrification study was carried out at 20 °C which is slightly below the optimum temperature (25 °C).

Grundmann et al. (1995) concluded that temperature had an obvious effect on the microbial activity, maximum nitrification rate. The maximum nitrification rate occurred at 25.5 °C in 0.0 to 20 cm soil and at 20 °C in 20 to 40 cm soil, suggesting an adaptation of soil nitrifying populations to the temperature regime of the soil.

Amin (1995) treated fresh soil samples of Darvel, Midelney, Darlieth and Freckenham soils with 100 mg N /g of soil; and incubated at -0.5 bar moisture potential and 0, 5, 10, 15 and 20 °C. He demonstrated that the nitrification rates increased as the temperature increased in all soils tested. The nitrification rates in Darvel, Freckenham and Darlieth soil samples indicated that the nitrifying bacteria can survive and can nitrify applied ammonium sulphate at temperatures down 0 °C.

Chen et al. (1998a) measured nitrification rates in the floodwater of an alkaline clay in the absence or presence of rice plants by inhibition of ammonium oxidation and ^{15}N-dilution techniques. They noted that nitrification rates in floodwater estimated by ^{15}N-dilution were generally higher than the rates estimated by the inhibitor method. In addition, the estimates of nitrification rates were generally higher during daylight hours than at night, and did not differ significantly between planted and unplanted pots.

In this study, the nitrification experiments were carried out by treating the soil with 100 mg NH_4-N /kg soil, incubating at -0.5 bar moisture potential and 20 °C, and determining extractable NH_4-N, NO_3-N and NO_2-N at intervals. Nitrification

usually gives a linear steady graph of nitrate formation so that linear regression can be used to calculate the nitrification rates.

Addiscott (1983) treated three fresh soil samples with ammonium chloride; and incubated at -0.05 bar moisture potential and 2.5, 5, 10, 15 and 20 °C. He reported that when the soils were incubated with added ammonium chloride, the increase in nitrate-N and the decline in ammonium-N were both linear with time, but were equal to each other in only one of the soils. These linear relationships did not reflect true zero-order kinetics because the rates of ammonium-N decline and nitrate-N production both depended on the initial ammonium concentration.

Flowers and O'Callaghan (1983) studied the effects of temperature, moisture content and the addition of pig slurry on nitrification in two soils. They pointed out that there was no accumulation of NO_2-N under the incubation conditions investigated but the accumulation of NO_3-N was linear for additions of 50 - 250 μg NH_4-N /g soil, either as ammonium sulphate or as pig slurry.

Some authors have used fresh soil samples in their nitrification and mineralization studies [Adisscott (1983), Flowers and O'Callghan (1983), Shah (1988), Mazumder (1992), Amin (1995), Grundmann et al. (1995), and Waggoner and Zuberer (1996)]. However, other authors have used air dried soil in their nitrification and mineralization studies [Ishaque and Cornfield (1972), Wickramasinhge et al. (1985), Burton et al. (1990), Dusek (1995), and Chen et al. (1998a)].

Amin (1995) treated fresh and dried and remoistened subsamples of Darvel, Dreghorn, Midelney, Darlieth and Freckenham series soils with 100 mg N /g of soil; and incubated at -0.5 bar moisture potential and 15 °C. He concluded that the fresh soil samples nitrified applied ammonium sulphate more quickly compared to air dried soil samples, and that the fresh soil samples showed a better reproducibility of nitrification rates and the data fitted better to a straight line. The air dried soil showed a curved pattern of nitrate accumulation with the rate of nitrate production increasing with time of incubation. It appears that air drying inhibited the nitrifying bacteria population which slowly recovered during incubation.

The soil samples used in the preliminary experiment and the nitrification experiment were fresh soils.

This chapter describes two nitrification experiments. The aim of the preliminary experiment was to compare the four soils [Intermediate Oxidation Plant (IOP) area (soil No. 1), H-Acid area (soil No. 9), Safety Fuse area (soil No. 11) and Nylon area (soil No. 15)] of the Ardeer site (see section 4.2.2.) with control soils (Darvel and Dreghorn soils) to get approximate nitrification rates in order to set up the main experiment.

The main experiment was carried out with the addition of ammonium-N contaminated groundwater to the soils [IOP area (soil No. 1), H-Acid area (soil No. 9), Darvel and Dreghorn soils] in order to :

1- Assess the effects of groundwater composition on the nitrification rate.

2-To determine the ability of soils to nitrify ammonium and thus promote leaching as nitrate.

5.2. MATERIALS AND METHODS

5.2.1. MATERIALS

5.2.1.1. SOIL SAMPLES

Four soil samples were collected from the Ardeer site. These four soil samples were as follows :

Soil sample from site No. 1 of the Intermediate Oxidation Plant (IOP) area.

Soil sample from site No. 9 of the H-Acid area.

Soil sample from site No. 11 of the Safety Fuse area.

Soil sample from site No. 15 of the Nylon area.

The locations of sampling points and description are given in section 4.2.2.1. These soil samples were selected according to the soil characterisation as described in section 4.3.2. and field observation of vegetation cover as described in section 4.2.2.1. which indicated that these soil samples seemed to be suitable for the nitrification and pot experiments.

Two further samples were taken of Darvel and Dreghorn series soils. These soil samples were chosen for the nitrification experiments as controls with good nitrification activity and differing texture and organic matter levels. Sampling sites for Darvel and Dreghorn series soils with a brief description are given below. These soil samples were attributed to Soil Series using the Soil Memoirs and Soil Maps for each area (Grant et al., 1962; and Ragg et al., 1976).

1- Darvel series soil :

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

2- Dreghorn series soil :

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

5.2.1.1.1. <u>SOIL SAMPLING</u>

The soil sampling and description for each soil are given below :

1- <u>Intermediate Oxidation Plant (IOP) area :</u>

The soil was taken from site No. 1 (Map 4.4.). The soil was taken in the fresh condition from the full depth of the topsoil (0 - 14 cm) since the topsoil is imported soil and well identified. The first 2 cm (turf grass) of the topsoil was not included in the sample.

2- <u>H-Acid area :</u>

The soil was taken from site No. 9 (Map 4.5.). The soil was taken in the fresh condition from the full depth of the topsoil (0 - 15 cm) since the topsoil is imported soil and well identified. The first 2 cm (turf grass) of the topsoil was not included in the sample.

3- <u>Safety Fuse area :</u>

The soil was taken from site No. 11 (Map 4.6.). The soil was taken in the fresh condition from the first 15 cm of the topsoil since the first 15 cm of the topsoil seemed to be a natural topsoil and after this 15 cm there were stones. The first 2 cm (turf grass) of the topsoil was not included in the sample.

4- Nylon area :

The soil was taken from site No. 15 (Map 4.7.). The soil was taken in the fresh condition from the first 20 cm of the topsoil since the first 20 cm of the topsoil seemed to be a natural topsoil. The first 2 cm (turf grass) of the topsoil was not included in the sample.

5- Darvel series soil :

The soil sample was taken from the top 15 cm.

6- Dreghorn series soil :

The soil sample was taken from the top 15 cm.

5.2.1.1.2. PREPARATION OF THE SOIL SAMPLES FOR THE NITRIFICATION EXPERIMENTS

The six soil samples were brought to the laboratory in labelled plastic bags and kept in the cold room at 2 °C until required for analysis and use in the nitrification and pot experiments.

Five kg soil was taken from each soil, spread on clean plastic sheet, the aggregates were broken to get the actual particle size, and each soil sample was mixed thoroughly to minimise the effects of local variations. Then each soil sample was partially air dried at room temperature just sufficiently to allow handling and to pass a 4 mm stainless steel sieve easily. The larger inert material is considered to have little effect on the chemical and nutritional status of the soil. A 4 mm sieve ensures as little disruption to the soil and micro-organisms as is possible. The micro-organisms could die in this stage and also more substrate could be made available to the microbes present due to disruption of the peds. The samples were then stored in the cold room at 2 °C until required for the nitrification experiments.

5.2.1.1.3. PHYSICAL AND CHEMICAL ANALYSIS OF THE SOIL SAMPLES

The physical and chemical analysis of the soil samples were carried out on the soil samples that passed a 2 mm stainless steel sieve which were used in the pot experiment (Chapter 6).

Moisture content was determined as described in section 2.7.

Organic matter (LOI) was determined as described in section 2.8.

pH was measured by a combined glass / reference electrode as in section 2.3.

The electrical conductivity was measured using a Jenway 4070 conductivity meter as described in section 2.5.

Soil moisture content at -0.5 bar soil moisture potential was determined as described in section 2.9.

Soil mechanical analysis was determined as described in section 2.10.

Inorganic nitrogen was extracted from the soil samples as described in section 2.17. NH_4-N was determined in the filtrate as described in section 3.1.3.2. using a standard series 0.0 to 1.0 mg /l NH_4-N in 0.5 M K_2SO_4 solution. NO_3-N was determined in the filtrate as described in section 2.11.1.3. using a standard series 0.0 to 1.0 mg /l NO_3-N in 0.5 M K_2SO_4 solution. NO_2-N was determined in the filtrate as described in section 2.11.1.3. using a standard series 0.0 to 1.0 mg /l NO_2-N in 0.5 M K_2SO_4 solution.

Phosphorus was extracted from the soil samples as described in section 2.18. Phosphorus was determined in the neutralised soil extract as described in section 2.11.2. using a standard series 0.0 to 1.0 mg P /l in 0.5 M $NaHCO_3$.

Potassium and magnesium were extracted from the soil samples as described in section 2.19. Potassium was determined in the filtrate by the Flame photometer as described in section 2.11.3. using a standard series 0.0, 5, 10, 15 and 20 mg K^+ /l in 1 M NH_4NO_3 solution. Magnesium was determined by the Atomic Absorption Spectrophotometer as described in section 2.11.6. using a standard series 0.0 to 0.5 and 0.0 to 2.0 mg Mg /l in 1 M NH_4NO_3 solution.

Cation exchange capacity (CEC) was determined as described in section 2.16.

5.2.1.2. GROUNDWATER WELLS

5.2.1.2.1. WATER SAMPLING

Three groundwater samples from wells No. TW 8, SW 11 and SW 15 were collected from the Ardeer site. These wells were chosen on the basis of the ammonium-N level and pH as described in section 4.3.1. These wells included wells with a moderate level of ammonium, wells No. SW 11 (Nylon area) and TW 8 (landfill area); and a well with very high level of ammonium, well No. SW 15 (in the middle of the Ardeer site).

The water samples were collected as described in section 4.2.1.1. The locations of the sampling points are given in section 4.2.1.1. and the description of these groundwater wells is given in section 4.2.1.2. The water samples were stored in the cold room at 2 °C until required for analysis and use in the nitrification experiments.

5.2.1.2.2. CHEMICAL ANALYSIS OF THE GROUNDWATER SAMPLES

The following water samples needed filtration and digestion :

Wells No. SW 11, TW 8 and SW 15.

These water samples were filtered and digested as described in section 4.2.1.3.

The following analysis were carried out as described in section 4.2.1.3. :

pH, EC, soluble ions such as NH_4-N, NO_3-N, NO_2-N and Cl and total metals (calcium, magnesium, sodium, potassium and iron).

In addition, copper, cadmium, chromium, nickel, zinc, lead, boron, barium, manganese and potassium were determined in the water samples by the Inductively Coupled Plasma (ICP) Spectroscopy as described in section 2.14.

5.2.2. NITRIFICATION EXPERIMENTS

5.2.2.1. PRELIMINARY EXPERIMENT :

NITRIFICATION RATE IN SOILS TREATED WITH AMMONIUM SULPHATE AT 100 mg NH_4-N /kg SOIL

5.2.2.1.1. EXPERIMENTAL DESIGN

Six soils [Intermediate Oxidation Plant (IOP) area (soil No. 1), H-Acid area (soil No. 9), Safety Fuse area (soil No. 11), Nylon area (soil No. 15), Darvel soil and Dreghorn soil] were incubated at -0.5 bar moisture potential and 20 °C, and treated with 100 mg NH_4-N /kg soil. The experiment was carried out with four replicates for each soil.

5.2.2.1.2. REAGENTS

PREPARATION OF 5000 mg /l NH_4-N SOLUTION :

Ammonium sulphate was dried at 105 °C for 1 hour and cooled in a desiccator for 30 minutes. 2.3585 g of dry ammonium sulphate was dissolved in approximately 90 ml deionised water in 100 ml volumetric flask and the volume was made to 100 ml with deionised water. The solution was stored at 2 °C.

5.2.2.1.3. INCUBATION PROCEDURE

A sample of fresh soil equivalent to 100 g (oven dry weight basis) was weighed into a 16 ounce glass bottle which was left open to permit aeration. Each soil sample was treated with 100 mg NH_4-N /kg soil by adding 2 ml of ammonium sulphate solution containing 5000 mg /l NH_4-N on the soil surface using a bulb pipette. 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 deionised water with a Pasteur pipette. The each soil sample was again mixed thoroughly using a spatula.

The glass bottle containing the sample was allowed to stand in the cold room at 2 °C for overnight. The low temperature helped the diffuse of ammonium in soil,

consequently ammonium was distributed well. Subsamples of each soil equivalent to 2.5 g (oven dry weight basis) were taken from the bottles for measuring extractable nitrogen at day zero. After taking a sample for measuring extractable nitrogen at day zero, the glass bottles were then placed in two large plastic containers in a randomised block design. Two replicates of each soil sample were placed in one container and the other two replicates of the same soil were placed in the other container to avoid the variability which may occur in one container and not in the other. This plastic container was lined with wetted filter paper to encourage evaporation of water and water saturated atmosphere. In addition, the container 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 lid of the plastic container was also lined with filter paper and tightly sealed to prevent water loss. The plastic container was large enough to ensure that there was sufficient oxygen for the incubation period.

The plastic containers were incubated at 20 °C. To determine the change in the ammonium-N, nitrate-N and nitrite-N, subsamples of each soil equivalent to 2.5 g (oven dry weight basis) were taken from the bottles at several time intervals. The duration of the time intervals varied with the soil types. Before taking a sample for measuring the extractable nitrogen, care was taken to readjust any loss in weight of the soil in the bottle by adding an appropriate weight of deionised water with a Pasteur pipette. The plastic containers were allowed to stand for 10 - 15 minutes to replenish the air at the end of each interval. The soil subsamples were extracted for measuring the concentration of NH_4-N, NO_3-N and NO_2-N. The soil samples were incubated until complete conversion of NH_4-N into NO_3-N.

5.2.2.1.4. EXTRACTION OF INORGANIC NITROGEN

NH_4-N, NO_3-N and NO_2-N were extracted as described in section 2.17. by shaking 2.5 g (oven dry weight basis) of the incubated soil with 50 ml of 0.5 M K_2SO_4 solution for 2 hours at 2 °C. The suspension was filtered through Whatman No. 2 filter paper. NH_4-N, NO_3-N and NO_2-N were determined in the filtrate. The blank determination was carried out by measuring NH_4-N, NO_3-N and NO_2-N in four blanks.

5.2.2.1.5. DETERMINATION OF INORGANIC NITROGEN

NH_4-N was determined in the filtrate as described in section 3.1.3.2. using standard series 0.0 to 1.0 and 0.0 to 5.0 mg /l NH_4-N in 0.5 M K_2SO_4 solution. NO_3-N was determined in the filtrate as described in section 2.11.1.3. using standard series 0.0 to 5.0 and 0.0 to 10 mg /l NO_3-N in 0.5 M K_2SO_4 solution. NO_2-N was determined in the filtrate as described in section 2.11.1.3. using standard series 0.0 to 1.0 mg /l NO_2-N in 0.5 M K_2SO_4 solution.

5.2.2.2. NITRIFICATION EXPERIMENT :

NITRIFICATION RATE IN SOILS TREATED WITH GROUNDWATER AND AMMONIUM SULPHATE AT 100 mg NH_4-N /kg SOIL

5.2.2.2.1. EXPERIMENTAL DESIGN

Four soils [Intermediate Oxidation Plant (IOP) area (soil No. 1), H-Acid area (soil No. 9), Darvel soil and Dreghorn soil] were incubated at -0.5 bar moisture potential and 20 °C, and treated with the following treatments :

Control treatment : 2500 mg /l NH_4-N solution.

Treatment No. 1 : Concentrated well water No. TW 8 contains 2560 mg /l NH_4-N.

Treatment No. 2 : Concentrated well water No. SW 11 contains 2490 mg /l NH_4-N.

Treatment No. 3 : Diluted well water No. SW 15 contains 2510 mg /l NH_4-N.

A randomised block design was used in this experiment and was replicated four times with the four treatments for each soil making a total of 64 incubations.

5.2.2.2.2. REAGENTS

5.2.2.2.2.1. CONTROL TREATMENT (2500 mg /l NH_4-N SOLUTION)

Ammonium sulphate was dried at 105 °C for 1 hour and cooled in a desiccator for 30 minutes. 0.5896 g of dry ammonium sulphate was dissolved in

approximately 40 ml deionised water in 50 ml volumetric flask and the volume was made to 50 ml with deionised water. The solution was stored at 2 °C.

5.2.2.2.2.2. CONCENTRATING GROUNDWATER

(a)- PREPARATION OF TREATMENT SOLUTION BY CONCENTRATING GROUNDWATER

It was planned to concentrate well waters No. TW 8 containing 39.8 mg /l NH_4-N and SW 11 containing 58.2 mg /l NH_4-N by evaporation to get groundwater solutions containing 5000 mg /l NH_4-N for treating soil prior to incubation. Well water No. SW 15 (4600 mg /l NH_4-N) did not require concentration.

A test sample of 1 litre of well water No. TW 8 with pH adjusted to 5.0 was evaporated without filtration to 10 ml on a hot plate stirrer in three stages. At each stage , the pH was checked and the crystalline material was removed by filtration.

NH_4-N was determined as described in section 3.1.3.2. using a standard series 0.0 to 10 mg /l NH_4-N in deionised water. NO_3-N was determined as described in section 2.11.1.3. using a standard series 0.0 to 10 mg /l NO_3-N in deionised water.

(b)- PREPARATION OF TREATMENT SOLUTION BY CONCENTRATING GROUNDWATER AND ADDITION OF AMMONIUM-N

As a result of the failure of the previous test, it was decided to concentrate the well waters No. TW 8 and SW 11 by a factor of 1 : 20 without filtration and add ammonium sulphate to provide 2500 mg /l NH_4-N.

PROCEDURE :

A 1 litre of each well water was placed into 2 litre beakers. The pH was adjusted to pH 6.0 using 0.5 M H_2SO_4 solution. The beaker was placed on a hot plate stirrer and left until the volume becomes about 40 ml. The concentrated well water was cooled to room temperature, then transferred by deionised water to 4 ounce glass bottle which has a mark at 50 ml and the volume was made to the mark with deionised water.

Then the bottle was shaken well. A sample of each concentrated well water was diluted in duplicate by a factor of 1 : 100 for analysis.

Well water No. SW 15 was diluted to get 2500 mg /l NH_4-N in it as follows : 30 ml of well water No. SW 15 was made up to volume with deionised water in a 50 ml volumetric flask. The flask was shaken well. For analysis, this diluted well was diluted again in duplicate by a factor of 1 : 250.

Ammonium was determined using automated coloriemetry with gas phase dialysis as described in section 3.3.2.2.3. using a standard series 0.0 to 10 mg /l NH_4-N in deionised water.

0.4868 g of ammonium sulphate (dried at 105 °C for 1 hour) was added to 50 ml of the concentrated well water No. TW 8 and 0.4245 g of ammonium sulphate was added to 50 ml of the concentrated well water No. SW 11. Then the bottles were shaken well.

As a final check on the concentration, a sample of each concentrated well water containing the addition of ammonium sulphate and the diluted well water No. SW 15 were diluted in duplicate by a factor of 1 : 250 for analysis.

5.2.2.2.3. <u>INCUBATION PROCEDURE</u>

A sample of fresh soil equivalent to 50 g (oven dry weight basis) was weighed into a 8 ounce glass bottle which was left open to permit aeration. Each soil sample was treated with the following treatments :

(a)- 2 ml of 2500 mg /l NH_4-N (control treatment) by a bulb pipette on the soil surface.

(b)- 2 ml of the concentrated well water No. TW 8. This treatment was added as a weight (2.12 g) which is equivalent to 2 ml since this treatment had much of iron oxide and could not be added as a 2 ml volume because the iron oxide would plug the pipette.

(c)- 2 ml of the concentrated well water No. SW 11 on the soil surface by a bulb pipette.

(d)- 2 ml of the diluted well water No. SW 15 on the soil surface by a bulb pipette.

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 deionised water with a Pasteur pipette. Then each soil sample was again mixed thoroughly using a spatula.

The glass bottle containing the sample was allowed to stand in the cold room at 2 °C for overnight. The low temperature helped the diffuse of ammonium in soil, consequently ammonium was distributed well. Subsamples of each soil equivalent to 2.5 g (oven dry weight basis) were taken from the bottles for measuring extractable nitrogen at day zero. After taking a sample for measuring extractable nitrogen at day zero, the glass bottles were then placed in four large plastic containers in a randomised block design. Four replicates of each soil sample were placed separately as one replicate in each container to avoid the variability which may occur in one container and not in the other three containers. This plastic container was lined with wetted filter paper to encourage evaporation of water and water saturated atmosphere. In addition, the container 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 lid of the plastic container was also lined with filter paper and tightly sealed to prevent water loss. The plastic container was large enough to ensure that there was sufficient oxygen for the incubation period.

The plastic containers were incubated at 20 °C. To determine the change in the ammonium-N, nitrate-N and nitrite-N, subsamples of each soil equivalent to 2.5 g (oven dry weight basis) were taken from the bottles at several time intervals. The duration of the time intervals varied with the soil types.

Before taking a sample for measuring the extractable nitrogen, care was taken to readjust any loss in weight of the soil in the bottle by adding an appropriate weight of deionised water with a Pasteur pipette. The plastic containers were allowed to stand for 10 - 15 minutes to replenish the air at the end of each interval. The soil subsamples were extracted for measuring the concentration of NH_4-N, NO_3-N and NO_2-N. The soil samples were incubated until complete conversion of NH_4-N into NO_3-N.

5.2.2.2.4. EXTRACTION OF INORGANIC NITROGEN

NH_4-N, NO_3-N and NO_2-N were extracted as described in section 5.2.2.1.4.

5.2.2.2.5. DETERMINATION OF INORGANIC NITROGEN

NH_4-N was determined in the filtrate as described in section 3.1.3.2. using standard series 0.0 to 5.0 mg /l NH_4-N in 0.5 M K_2SO_4 solution. NO_3-N was determined in the filtrate as described in section 2.11.1.3. using standard series 0.0 to 10 mg /l NO_3-N in 0.5 M K_2SO_4 solution. NO_2-N was determined in the filtrate as described in section 2.11.1.3. using standard series 0.0 to 1.0 mg /l NO_2-N in 0.5 M K_2SO_4 solution.

5.2.2.2.6. STATISTICAL ANALYSIS AND DATA HANDLING

The measured values of NH_4-N, NO_3-N, NO_2-N and total nitrogen were plotted on graphs by using the Microsoft Excel Package (Version 5.0).

Analysis of variance and regression to determine rate of nitrification were carried out by using Minitab package (Release 9.2).

A t-test was carried out by using Minitab package (Release 9.2) to test the difference between the rate of ammonium loss and the rate of nitrate formation using the twosamples command with pooled subcommand.

5.3. RESULTS AND DISCUSSION

5.3.1. CHEMICAL AND PHYSICAL PROPERTIES OF THE SOIL SAMPLES

The textural properties of the soil samples are presented in Table 5.1. The results are the mean of three replicates. As can be seen, the Darvel, Dreghorn, Intermediate Oxidation Plant (IOP), Safety Fuse and Nylon soils were sandy soils, while the H-Acid soil was a clayey soil. The IOP, H-Acid and Safety Fuse areas have imported topsoil, while the Nylon area is sandy with some development of a natural topsoil.

Table 5.1. : Textural properties of the soil samples.

Soil sample	Coarse + medium sand (%)	Fine sand (%)	Silt (%)	Clay (%)
Darvel	32.6	22.0	24.8	22.9
Dreghorn	77.7	14.2	4.1	4.4
IOP soil	67.7	16.7	7.8	6.7
H-Acid soil	7.3	7.8	35.2	54.6
Safety Fuse soil	70.2	21.1	4.8	3.1
Nylon soil	71.5	21.2	3.4	2.3

The chemical and physical characteristics of the soil samples are given in Table 5.2. The results are the mean of three replicates. The pH of the Darvel, Dreghorn, IOP and Nylon soils was approximately neutral, however, the pH of the H-Acid and Safety Fuse soils was slightly acidic. Overall, the electrical conductivity was very low in all six soils. The moisture content, organic matter content and CEC were higher in the Darvel and H-Acid soils than the other four soils.

Table 5.2. : Chemical and physical characteristics of the soil samples.

Soil sample	pH	EC* (ms /cm)	Moisture content at -0.5 bar (%)	Organic matter (%)	CEC (c $mole_c$ /kg)
Darvel	7.2	0.11	32.7	11.7	32.4
Dreghorn	7.2	0.10	17.7	5.2	14.3
IOP soil	6.8	0.21	13.0	4.4	14.3
H-Acid soil	6.0	0.14	51.7	18.2	59.1
Safety Fuse soil	5.9	0.27	8.6	2.9	8.7
Nylon soil	6.9	0.09	5.5	1.9	7.1

* EC = Electrical conductivity.

5.3.2. PRELIMINARY EXPERIMENT :

NITRIFICATION RATE IN SOILS TREATED WITH AMMONIUM SULPHATE AT 100 mg NH_4-N /kg SOIL

A preliminary experiment was carried out to evaluate the four soils [Intermediate Oxidation Plant (IOP) area (soil No. 1), H-Acid area (soil No. 9), Safety Fuse area (soil No. 11) and Nylon area (soil No. 15)] of the Ardeer site plus two control soils (Darvel and Dreghorn soils) for their nitrification rate. Approximate nitrification rates were required in order to set up a nitrification experiment to examine nitrification of ammonium applied as well water.

Six soils were treated with 100 mg NH_4-N /kg soil, incubated at -0.5 bar moisture potential and 20 °C, and extractable NH_4-N, NO_3-N and NO_2-N were determined at intervals. The measured quantities of ammonium, nitrate and nitrite were added together to give total inorganic nitrogen and graphs of ammonium, nitrate, nitrite and total inorganic nitrogen were plotted against time. The nitrification of ammonium sulphate added to the soil samples during incubation at -0.5 bar moisture potential and 20 °C are illustrated in Figures 5.1., 5.2., 5.3., 5.4., 5.5. and 5.6. The incubation period varied with the soil type, however, the figures for all the soils were made on the same scale for easy comparison. Typical data for single incubations are shown in the figures.

The four replicates of each soil showed good agreement and the graphs showed a high degree of linearity. The nitrogen balance is indicated by the total nitrogen value. The Intermediate Oxidation Plant (IOP) and Safety Fuse soils showed a slight decrease in total nitrogen at the beginning of incubation. This could be due to immobilisation, ammonia volatilisation or ammonium fixation but since these soils are extremely sandy with neutral pH it is more likely to be immobilisation. The Darvel and H-Acid soils showed low rates of mineralization. Nevertheless, total inorganic nitrogen showed good agreement between different extraction dates. As can be seen, the applied ammonium disappeared in approximately 10 days in the Darvel and Dreghorn soils, and in approximately 34 days in the IOP and H-Acid soils. There was only a very slight decrease in the applied ammonium in the Safety Fuse and Nylon soils.

The IOP and Dreghorn soils showed a slight initial lag before the maximum rate of nitrification was achieved, whereas the Darvel, H-Acid and Nylon soils showed no lag period. There was then a phase where ammonium decreased and nitrate increased linearly with time, after this the nitrate formation and ammonium loss rates declined as the ammonium substrate became limiting.

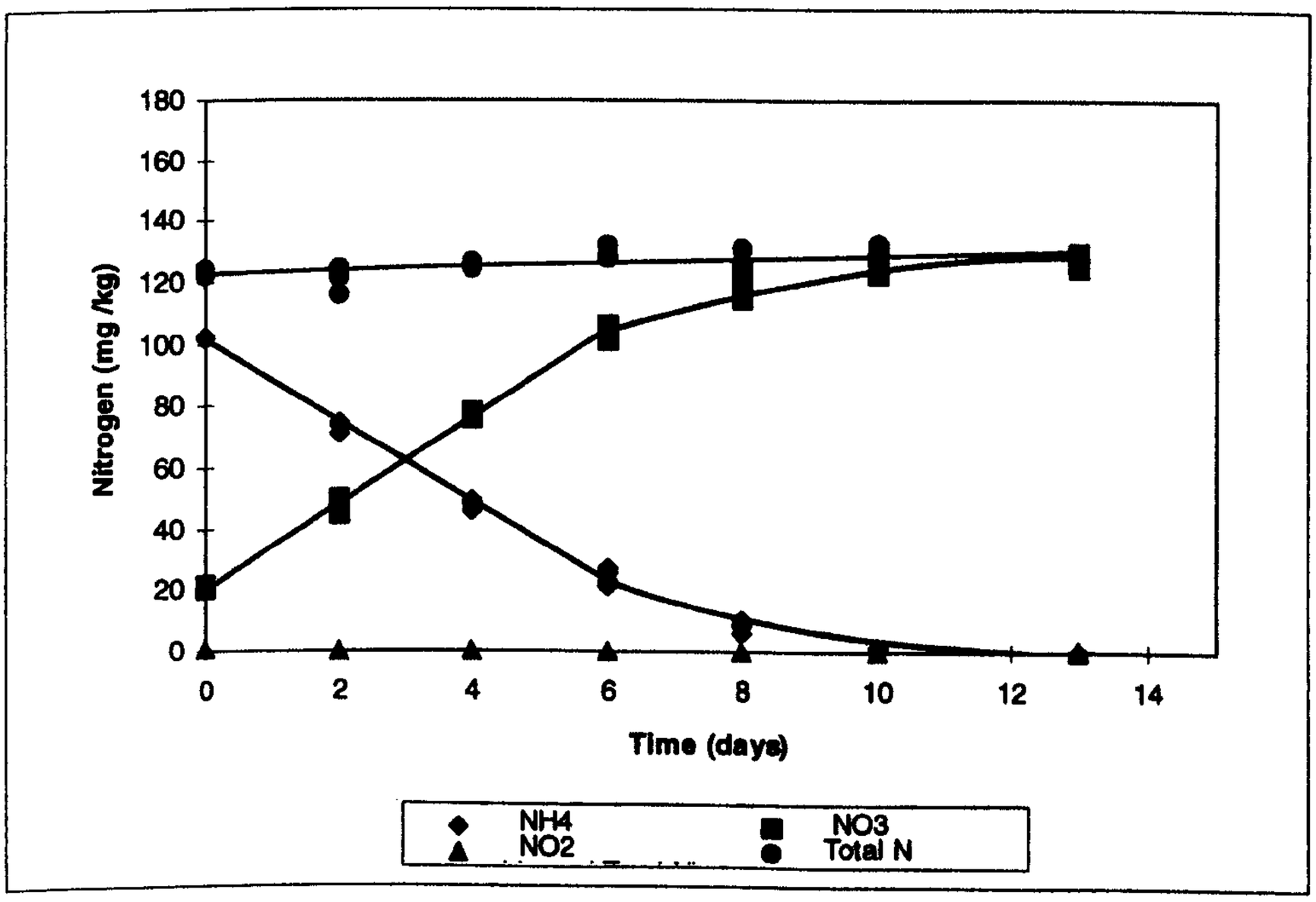


Figure 5.1. : Nitrification in Darvel soil treated with 100 mg NH_4-N /kg soil and incubated at -0.5 bar moisture potential and 20 °C.

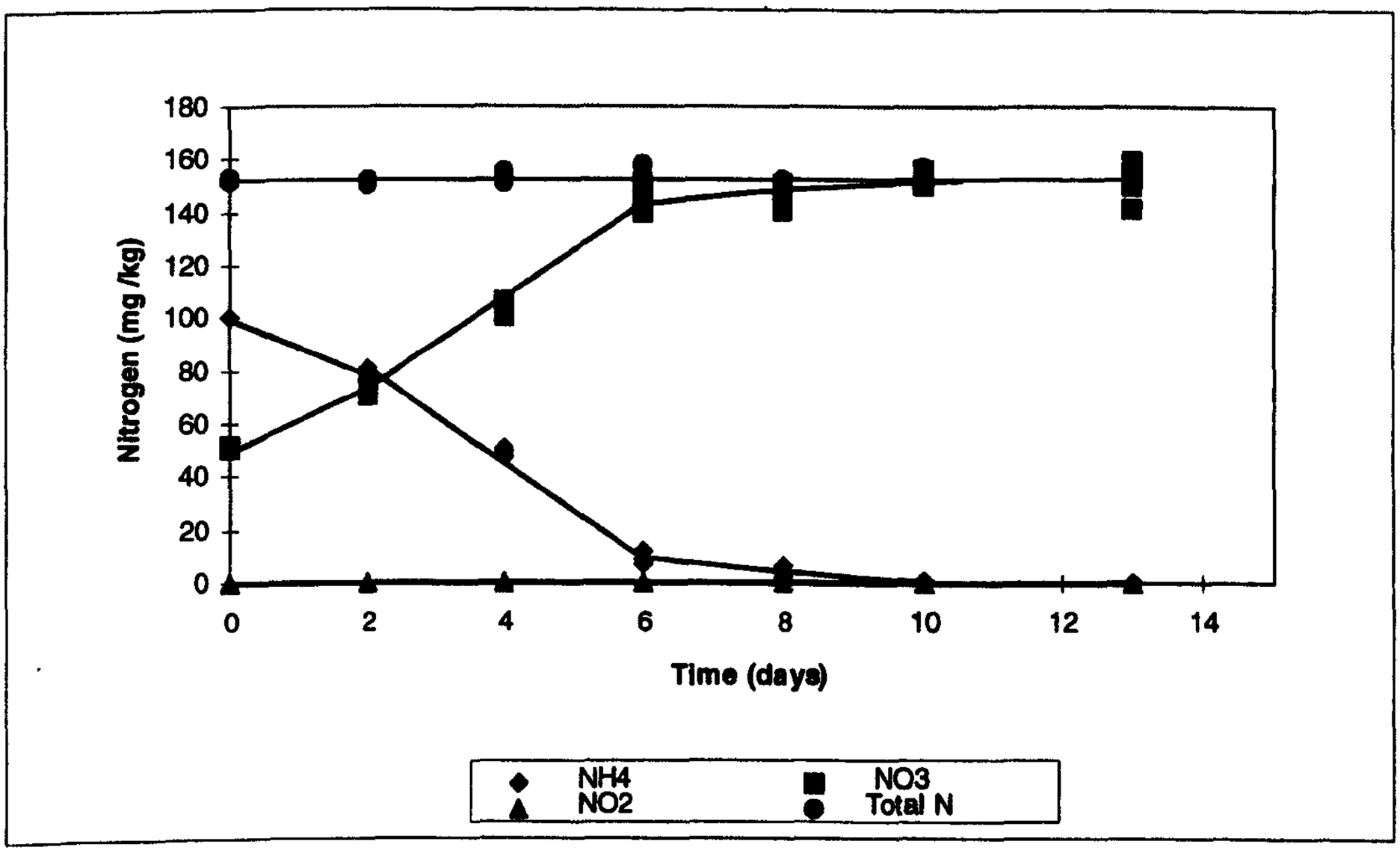


Figure 5.2. : Nitrification in Dreghorn soil treated with 100 mg NH_4-N /kg soil and incubated at -0.5 bar moisture potential and 20 °C.

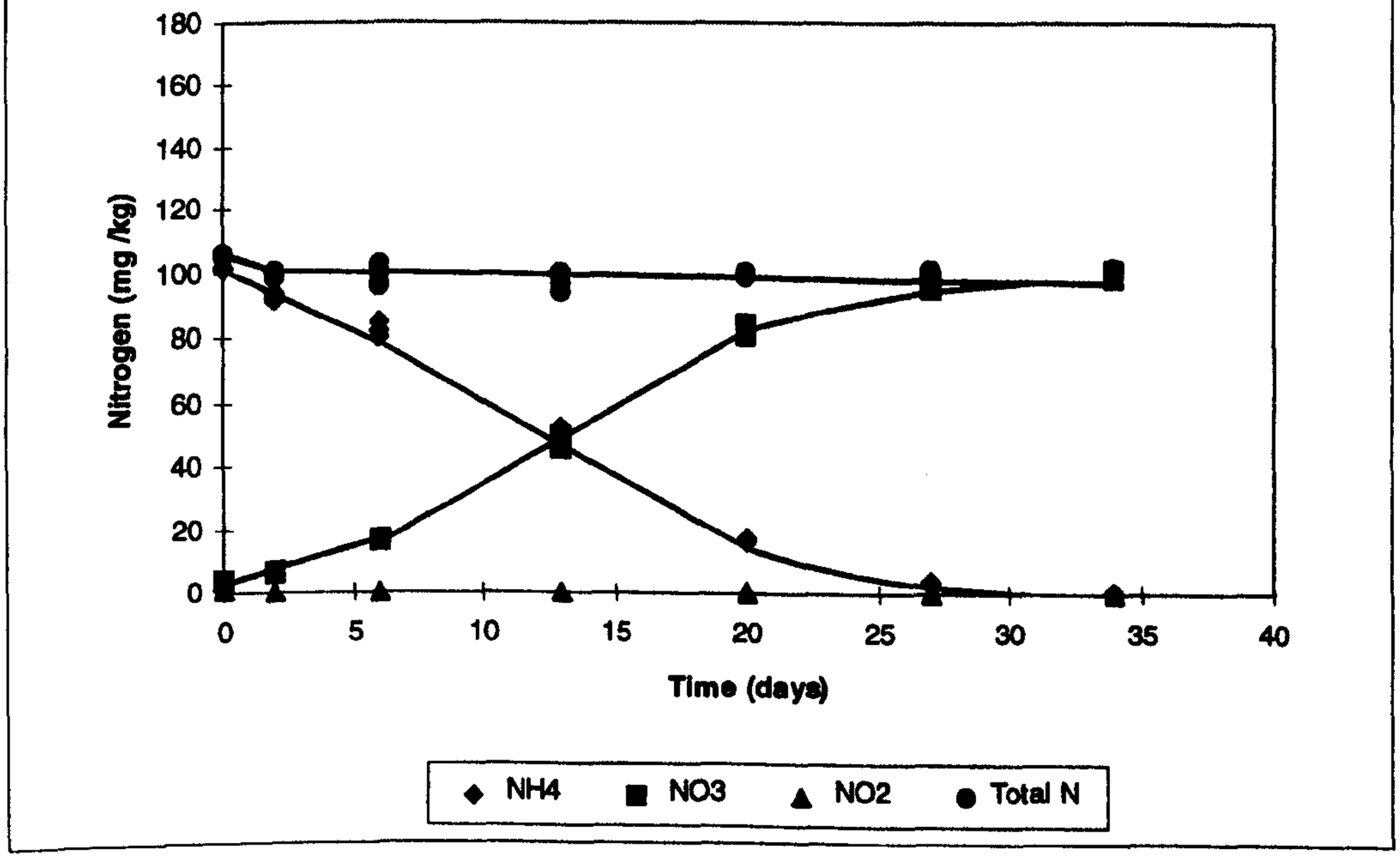


Figure 5.3. : Nitrification in the IOP soil treated with 100 mg NH_4-N /kg soil and incubated at -0.5 bar moisture potential and 20 °C.

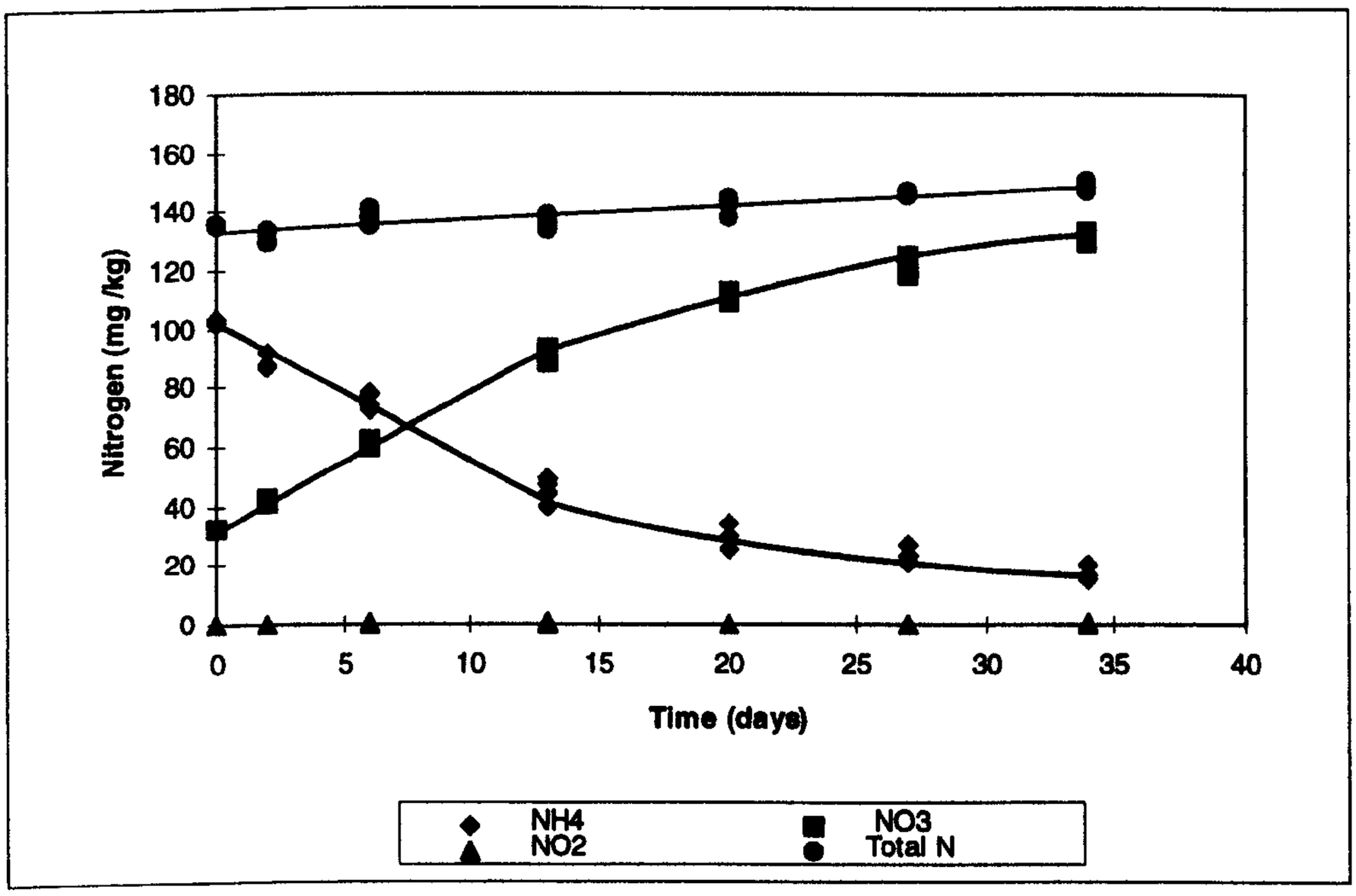


Figure 5.4. : Nitrification in the H-Acid soil treated with 100 mg NH_4-N /kg soil and incubated at -0.5 bar moisture potential and 20 °C.

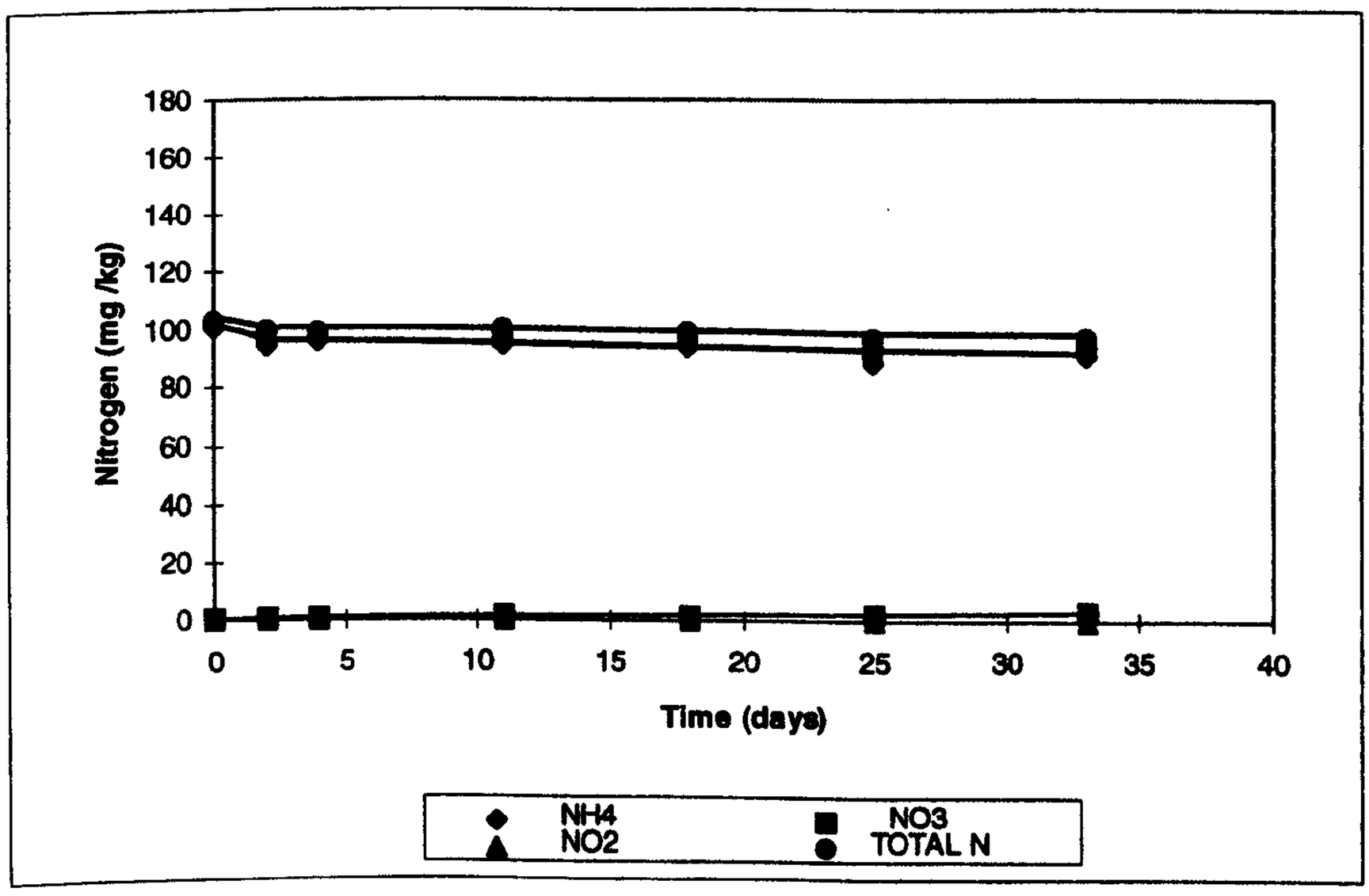


Figure 5.5. : Nitrification in the Safety Fuse soil treated with 100 mg NH_4-N /kg soil and incubated at -0.5 bar moisture potential and 20 °C.

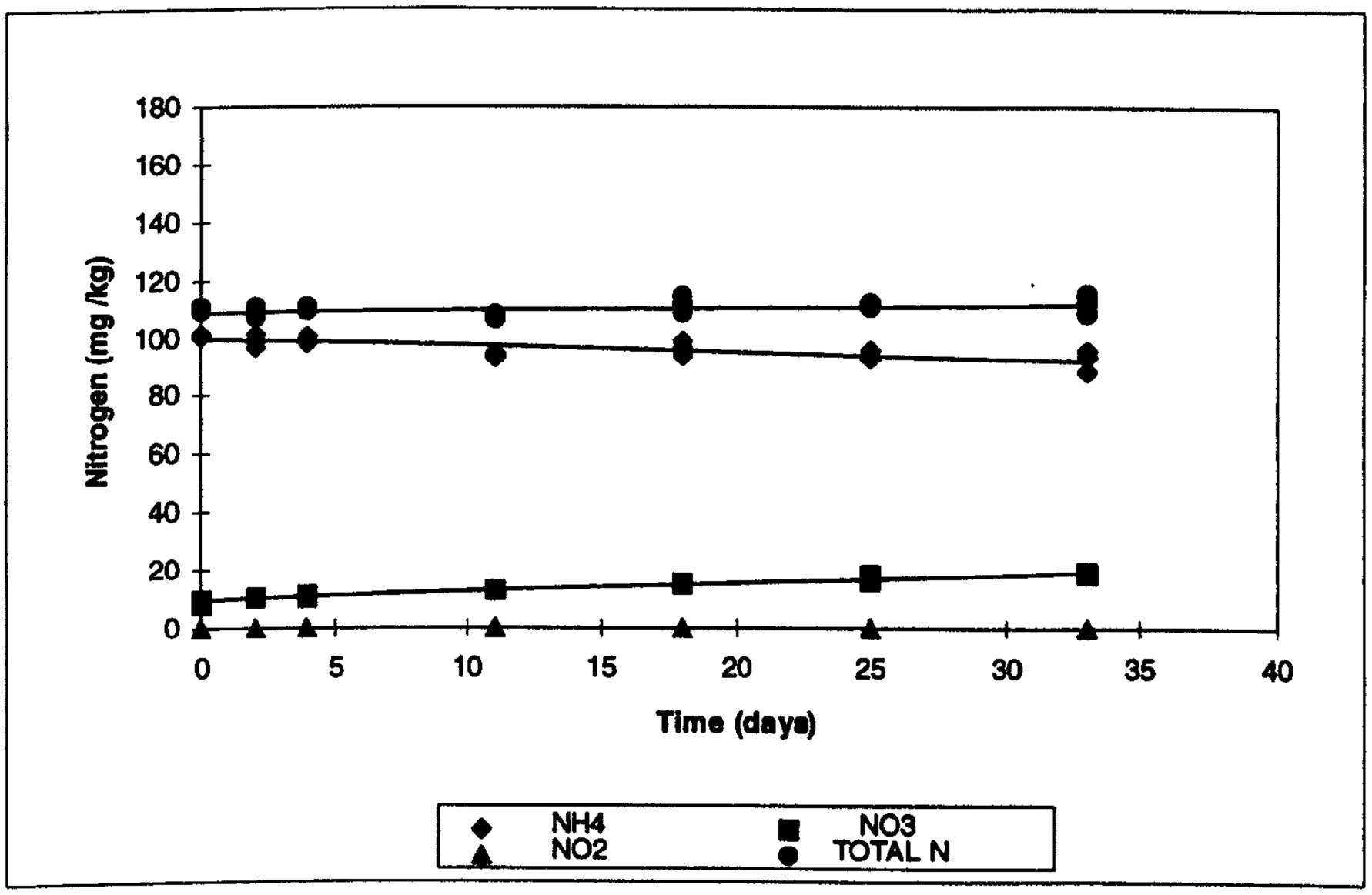


Figure 5.6. : Nitrification in the Nylon soil treated with 100 mg NH_4-N /kg soil and incubated at -0.5 bar moisture potential and 20 °C.

The linear period where the increase in nitrate concentration and decrease in ammonium concentration occurred concurrently was used to calculate the nitrification rate. Care was taken to avoid the initial part of the graph where immobilisation or a lag period were a problem and the last part where the ammonium substrate was a limiting factor. Ammonium loss and nitrate formation were fitted to a straight line using linear regression to calculate the nitrification rates. Total inorganic nitrogen levels were also fitted to a straight line to calculate the mineralization rates.

Table 5.3. demonstrates nitrification rates based on loss of ammonium and formation of nitrate in the soil samples incubated at -0.5 bar moisture potential and 20 °C. As can be seen, the ammonium loss and nitrate formation rates were significantly higher in the Dreghorn and Darvel soils than those of the other four soils. The ammonium loss and nitrate formation rates were less in the Intermediate Oxidation Plant (IOP) and H-Acid soils, and much less in the Safety Fuse and Nylon soils. The ammonium loss and nitrate formation rates showed a decrease in the incubated soils in the following order :

Dreghorn < Darvel < IOP < H-Acid < Nylon < Safety Fuse.

The t-test analysis showed that the difference between ammonium loss and nitrate formation rates were very significantly different in the Darvel soil ($p < 0.01$) but not significant in the Dreghorn, IOP, H-Acid, Safety Fuse and Nylon soils ($p > 0.05$). In the Darvel soil, there was a greater rate of nitrate formation than ammonium loss and this could be because there was mineralisation occurring. The better measure of nitrification rate is the nitrate formation rate because ammonium loss rate was affected by ammonium-N input as shown in the Darvel soil.

Table 5.3. : Nitrification rates based on loss of ammonium and formation of nitrate in the soil samples incubated at -0.5 bar moisture potential and 20 °C.

Soil sample	Ammonium-N loss (mg /kg soil /day)			Nitrate-N formation (mg /kg soil /day)		
	Rate	Mean[1]	95 % C. I.	Rate	Mean[1]	95 % C. I.
Darvel soil :						
Replicate No. 1	-13.4			14.3		
Replicate No. 2	-13.1			14.0		
Replicate No. 3	-12.4			13.6		
Replicate No. 4	-12.8	-12.9	(-13.6, -12.3)	14.4	14.1	(13.5, 14.7)**
Dreghorn soil :						
Replicate No. 1	-17.8			19.1		
Replicate No. 2	-17.2			18.1		
Replicate No. 3	-16.8			17.3		
Replicate No. 4	-17.3	-17.3	(-17.9, -16.6)	17.5	18.0	(16.7, 19.3) NS
IOP soil (Soil No. 1) :						
Replicate No. 1	-4.5			4.6		
Replicate No. 2	-4.7			4.8		
Replicate No. 3	-4.6			4.8		
Replicate No. 4	-4.8	-4.7	(-4.9, -4.4)	4.6	4.7	(4.5, 4.9) NS
H-Acid soil (Soil No. 9) :						
Replicate No. 1	-4.8			4.7		
Replicate No. 2	-4.3			4.6		
Replicate No. 3	-4.2			4.3		
Replicate No. 4	-3.9	-4.3	(-4.9, -3.7)	4.4	4.5	(4.2, 4.8) NS
Safety Fuse soil (Soil No. 11) :						
Replicate No. 1	-0.3			0.1		
Replicate No. 2	-0.1			0.1		
Replicate No. 3	-0.1	-0.2	(-0.4, 0.0)	0.1	0.1	(0.1, 0.1) NS
Nylon soil (Soil No. 15) :						
Replicate No. 1	-0.3			0.3		
Replicate No. 2	-0.2			0.3		
Replicate No. 3	-0.2	-0.2	(-0.3, -0.1)	0.3	0.3	(0.3, 0.4) NS

1 = For each soil, mean rates which are significantly different using a t-test are indicated as follows :

NS = Not significant at $p > 0.05$.
* = Significant at $p < 0.05$.
** = Very significant at $p < 0.01$.
*** = Highly significant at $p < 0.001$.

A comparison of nitrification rates reported by other authors is shown in Table 5.4. It was difficult to find data at 20 °C, therefore, in any comparisons allowance must be made for the effect of temperature. The soil samples used in this preliminary experiment and the nitrification experiment were fresh soils. Since Amin (1995) concluded that fresh soil samples nitrified applied ammonium sulphate more quickly compared to air dried soil samples, only data for fresh soils are considered.

Table 5.4. Nitrification rates reported by several authors.

Author	Moisture condition	Temperature (°C)	Soil condition	Nitrification rate (mg N /kg soil /day)
Flowers and O'Callaghan (1983)	45 % Water Holding Capacity (WHC)	15 °C	Fresh	2.5 - 5.6
Addiscott (1983)	-0.05 bar moisture potential	15 °C 20 °C	Fresh Fresh	5.32 - 12.73 12.7
Mazumder (1992)	-0.5 bar moisture potential	25 °C	Fresh	10.5 - 17.48
Grundmann et al. (1995)	45 % Water saturation	20 °C	Fresh	1.99 - 3.34
Amin (1995)	-0.5 bar moisture potential	15 °C	Fresh	8.8 - 27.1
Waggoner and Zuberer (1996)	~ 25 % Gravimetric moisture content	30 °C	Fresh	2.6

The measured nitrification rates in the Darvel and Dreghorn soils are similar to the high end of the range in the table above. The Safety Fuse and Nylon soils are lower than any reported, while the Intermediate Oxidation Plant (IOP) and H-Acid soils are similar to the lower range reported.

The high nitrification rates in the Darvel and Dreghorn soils are possibly attributed to their being fertile, natural garden soils with favourable pH (7.2). These soils have been used in previous studies. Amin (1995) incubated fresh soil samples of the Darvel and Dreghorn at -0.5 bar moisture potential and 15 °C. He reported that applied ammonium disappeared in approximately a week in the Darvel soil but nitrification was slower in the Dreghorn soil. The nitrification rates were 17.6 mg N /kg soil /day in the Darvel soil and 8.8 mg N /kg soil /day in the Dreghorn soil. Mazumder (1992) incubated fresh soil samples of the Darvel and Dreghorn at -0.5 bar moisture potential and 25 °C. He pointed out that the Darvel soil nitrified faster than expected. The nitrification rates were 17.48 mg N /kg soil /day in the Darvel soil and 10.5 mg N /kg soil /day in the Dreghorn soil. Compared to these authors, the nitrification rate measured in the current experiment is lower in the Darvel soil and is higher in the Dreghorn soil but of a similar order.

Of the factors likely to affect the nitrification rate, the pH range is reasonable in all soils with the exception of the H-Acid and Safety Fuse soils. The low nitrification rates in the H-Acid and Safety Fuse soils may be due to their low pH (6.0 and 5.9, respectively). Burton et al. (1990) attributed the decline in rate of net NO_3-N production between weeks 4 and 8 of incubation to the development of unfavourable conditions such as lower pH and possibly a reduction in oxygen supply in sludge-dominated microsites. However, this is opposite to the results of Ishaque and Cornfield (1972) who attributed the occurrence of nitrification at low pH to the ability of acid-adapted strains of autotrophic nitrifying bacteria to function under this condition.

Shah (1988) pointed out that nitrification rates of added ammonium (100 mg N / kg soil) in coal mine soil samples was highly pH dependent, being inhibited below pH 4.8. However, even on some sites above this pH there was no measurable nitrification.

Inhibition of nitrification can occur due to high levels of ammonium. Flowers and O'Callaghan (1983) pointed out that the nitrification rate constant in soils treated with 50 µg NH_4-N /g soil was not significantly affected ($p = 0.05$) by the form of ammonium added. Addition of 250 µg NH_4-N as ammonium sulphate caused a marked inhibition of nitrification at all moisture contents and temperature but addition of 250

μg NH_4-N as pig slurry caused a marked increase in nitrification rate, the increase was greater at the higher temperatures and moisture contents. Mauret et al. (1996) studied the specific effects and the possible interactions between initial ammonia concentration, pH and temperature on the nitrification reactions, ammonium oxidation and nitrite oxidation, as well as on the experimental conditions leading to nitrite build-up. They summarised that the ammonium oxidation rate increases by roughly 40 % from 15 to 25 °C, and only increases by 24 % on average from pH 7.0 to 8.5. A high $(NH_4)_o$ and a pH of 8.5 cause an inhibition of the nitrite oxidation and a partial nitrite build-up amounting to 50 % of the initial quantity of ammonium introduced. Therefore, the effect of high concentration in NH_3 on the nitrite oxidation is stressed. The threshold of the beginning of Nitrobacter inhibition is situated between 6.6 and 8.9 mg N-NH_3 /l.

Fdz-Polanco et al. (1994) pointed out that the population and activity of *Nitrobacter* depend on the specific concentration of free ammonia / VAS (Volatile Attached Solids). Its activity decreases heavily for values above 1 mg N-NH_3 /mg VAS, which is taken as the inhibition threshold value, and increases exponentially under this threshold value. Fdz-Polanco et al. (1996) showed that the free ammonia inhibition effect highly depends on the values of pH, temperature and ammonium concentration. For instance, in situations of free ammonia inhibition, the combined effect of temperature, pH and ammonium concentration bring about different nitrite accumulations for the same specific free ammonia concentration, which may explain the differences found in the literature for the thresholds of free ammonia inhibition established by many authors. Therefore, any inhibition situation should be defined by the values of free ammonia, ammonium and biomass concentrations, pH and temperature. In conditions of no free ammonia inhibition and low values of temperature and pH, high ammonium concentrations bring about a higher relative activity of ammonia oxidiser micro-organisms and then, a nitrite accumulation may happen in the system.

Although this inhibition is most likely to occur in very sandy soils, it is unlikely to be the reason for the very low nitrification in the Safety Fuse and Nylon soils.

Toxic metals may inhibit nitrification rate. Harper et al. (1996) pointed out that chromium (Cr^{+3}) and nickel (Ni^{+2}), at soluble concentrations of approximately 0.30 and 0.70 mg /l, respectively, caused inhibitory effects to the nitrification-denitrification system. Chromium affected both the nitrification and denitrification processes, but nickel impaired only the nitrification performance.

Dusek (1995) concluded that additions of both 100 and 500 mg Cd /kg dry soil significantly lowered the ability of both soils to nitrify 100 µg added NH_4-N /g dry soil as a substrate, and consequently resulted in a decreased rate of nitrate formation (maximum inhibition obtained was 60 % in a calcareous soil and 45 % in a non-calcareous soil). However, the addition of 10 mg Cd /kg dry soil intensified N-mineralization in both soils, probably as a result of a higher concentration of readily metabolised substrate originating from killed bacteria or fungi. The harmful effect of cadmium was more pronounced in the calcareous soil, probably due to the higher sensitivity of nitrite oxidisers in these soil samples. Lee et al. (1997) concluded that the nitrifying bacteria were more sensitive to copper than nickel, in general; and *Nitrosomonas* sp. was more sensitive to copper and nickel than *Nitrobacter* sp., in particular. *Nitrosomonas* sp. was gradually inhibited by copper while there appears to be a threshold nickel concentration at which the activity of *Nitrosomonas* sp. was severely inhibited. For example, a high influent of nickel concentration of 50 mg /l was needed to cause a similar percent inhibition of ammonium oxidation to a copper concentration of 5 mg /l. Grunditz et al. (1998) tested the effect of heavy metals (Cr, Ni, Cu, Zn, Pb and Cd) on ammonium oxidation and nitrite oxidation in industrial wastewater in pure culture. They reported that ammonium oxidation was most influenced by the concentration of zinc and copper while nitrite oxidation was most influenced by concentration of zinc and nickel.

The low nitrification rate in the H-Acid soil may be due to its high level of nickel (92 mg Ni /kg) which exceeds the ICRCL Guideline Note 59 /83 (1987) threshold value.

The age of the area may also have an effect on nitrification as the nitrification on disturbed sites increases with the age of the area. The IOP, H-Acid and Safety Fuse soils were treated with imported topsoil which was added in Autumn 1996 for the IOP

and Safety Fuse areas and in Autumn 1993 for the H-Acid area. The Nylon site is not topsoiled (natural soil) and the Nylon plant was demolished in 1981 allowing little time for soil development. Waggoner and Zuberer (1996) conducted a field study to determine nitrification potentials, enumerate nitrifying bacteria and to determine the relationship between the two in mixed-overburden. They found that nitrification potentials were lowest in fresh spoil and increased with age of site. Nitrification rates at the 1 year old site were equivalent to those at an adjacent unmined site.

Another factor affecting on the nitrification rate is the organic matter content in the soil as the organic nitrogen can be mineralized to ammonium-N which is important for the nitrifying bacteria. The organic matter content is low in the IOP, Safety Fuse and Nylon soils; and higher in the H-Acid soil. The IOP, Safety Fuse, Nylon and H-Acid soils were not fertilised and plant growth was generally poor. The nitrogen (NH_4-N and NO_3-N) levels were very low in the IOP, Safety Fuse and Nylon soils; and low in the H-Acid soil. The phosphorus levels were very low in the IOP soil, slightly higher in the H-Acid soil than the IOP soil, low in the Safety Fuse soil, and high in Nylon soil. The potassium levels were very low in the IOP soil, slightly higher in the H-Acid soil than the IOP soil, and low in the Safety Fuse and Nylon soils. In the H-Acid soil, there was more available N, P and K and this coincided with better vegetation [white clover mixed with grass (about 50 % : 50 % mix, full coverage)]. The vegetation of the IOP soil was white clover and very poor grass (about 50 % coverage). The vegetation of the Safety Fuse soil was mostly white clover and poor grass (80 % coverage). In the Nylon soil, there was reasonable coverage of legumes (Gorse). Low fertility, poor vegetation growth and low organic matter content in these soils may be expected to result in low mineralization rates and therefore, low levels of ammonium substrate to sustain the nitrifying bacteria.

Mineralization rates in the soil samples incubated at -0.5 bar moisture potential and 20 °C are summarised in Table 5.5. These mineralization rates were measured in a much shorter time than is normal, and there tends to be an increase rate of mineralization in the first few days of incubation (Khan, 1987). Therefore, the mineralization rates are not fully reliable. The mineralization rates were in the range of 0.0 to 0.6 mg N /kg soil /day. As can be seen, there was a small amount of

mineralization in the Darvel and H-Acid soils. The properties (high organic matter content, high CEC and high clay content) of the Darvel and H-Acid soils support their mineralization rates (0.6 mg N /kg soil /day in the Darvel soil and 0.5 mg N /kg soil /day in the H-Acid soil). There was no mineralization in the Dreghorn, IOP, Safety Fuse and Nylon soils.

Addiscott (1983) incubated three fresh soil samples at -0.05 bar moisture potential and 20 °C. He reported mineralization rates of 0.13 and 0.26 mg N /kg soil /day. Khan (1987) incubated nine fresh soil samples at -0.5 bar moisture potential and 10 °C. He reported mineralization rates ranged from 0.12 to 0.28 mg N /kg soil /day.

From this preliminary experiment, it was concluded that the Safety Fuse and Nylon soils had extremely low nitrification rates and were not suitable for further studies. The Intermediate Oxidation Plant (IOP) and H-Acid soils were suitable for subsequent work together with two garden soils (Darvel and Dreghorn) which were used for comparison.

Table 5.5. : Mineralization rates in the soil samples incubated at -0.5 bar moisture potential and 20 °C.

Soil sample	Mineralization rate (mg N /kg soil /day)		
	Rate	Mean	95 % C. I.
Darvel soil :			
Replicate No. 1	0.7		
Replicate No. 2	0.5		
Replicate No. 3	0.9		
Replicate No. 4	0.2	0.6	(0.1, 1.0)
Dreghorn soil :			
Replicate No. 1	0.4		
Replicate No. 2	-0.0		
Replicate No. 3	-0.1		
Replicate No. 4	-0.4	-0.0	(-0.6, 0.5)
IOP soil (Soil No. 1) :			
Replicate No. 1	0.1		
Replicate No. 2	0.1		
Replicate No. 3	0.2		
Replicate No. 4	0.0	0.1	(-0.0, 0.2)
H-Acid soil (Soil No. 9) :			
Replicate No. 1	0.4		
Replicate No. 2	0.4		
Replicate No. 3	0.5		
Replicate No. 4	0.5	0.5	(0.4, 0.5)
Safety Fuse soil (Soil No. 11) :			
Replicate No. 1	-0.3		
Replicate No. 2	-0.0		
Replicate No. 3	-0.1	-0.1	(-0.3, 0.1)
Nylon soil (Soil No. 15) :			
Replicate No. 1	0.1		
Replicate No. 2	0.1		
Replicate No. 3	0.1	0.1	(-0.0, 0.2)

5.3.3. NITRIFICATION EXPERIMENT :

NITRIFICATION RATE IN SOILS TREATED WITH GROUNDWATER AND AMMONIUM SULPHATE AT 100 mg NH_4-N /kg SOIL

Four soil samples [Intermediate Oxidation Plant (IOP) area (soil No. 1), H-Acid area (soil No. 9), Darvel soil and Dreghorn soil] were treated with three well waters to supply 100 mg NH_4-N /kg soil, incubated at -0.5 bar moisture potential and 20 °C, and extractable NH_4-N, NO_3-N and NO_2-N were determined at intervals.

5.3.3.1. WATER QUALITY PARAMETERS

The chemical composition of the groundwater samples is presented in Table 5.6. The metal content in the groundwater samples is given in Table 5.7. As can be seen, the pH was slightly high in all three wells. The well waters varied in the nitrogen, electrical conductivity and composition of the dissolved salts. The heavy metals were low in all well waters.

Table 5.6. : Chemical composition of the groundwater samples.

Well No.	pH	EC* (ms /cm)	NH_4-N (mg /l)	NO_3-N (mg /l)	NO_2-N (mg /l)	Cl (mg /l)
TW 8	7.3	4.7	39.8	9.9	0.4	1170
SW 11	8.2	0.7	58.2	0.2	< 0.1	25.4
SW 15	7.5	62	4600	5580	0.1	343

Well No.	K (mg /l)	Na (mg /l)	Ca (mg /l)	Mg (mg /l)	Fe (mg /l)
TW 8	53.8	126	520	297	95
SW 11	6.5	40.0	44.5	4.4	2.5
SW 15	350	1610	1380	1430	9.2

* EC = Electrical conductivity.

Table 5.7. : Metals content of the groundwater samples determined by the Inductively Coupled Plasma (ICP) Spectroscopy.

Well No.	Cu (mg /l)	Cd (mg /l)	Cr (mg /l)	Zn (mg /l)	Ni (mg /l)	Pb (mg /l)
TW 8	< 0.01	< 0.01	< 0.01	0.3	< 0.01	< 0.1
SW 11	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.1
SW 15	< 0.01	< 0.01	< 0.01	0.6	0.21	< 0.1

Well No.	B (mg /l)	Ba (mg /l)	Mn (mg /l)	K (mg /l)
TW 8	107	0.04	1.0	50.7
SW 11	0.1	0.01	0.1	4.5
SW 15	1.1	0.08	7.9	324

5.3.3.1.1. CONCENTRATING GROUNDWATER

5.3.3.1.1.1. PREPARATION OF TREATMENT SOLUTION BY CONCENTRATING GROUNDWATER

It was planned to concentrate well waters No. TW 8 containing 39.8 mg /l NH_4-N and SW 11 containing 58.2 mg /l NH_4-N by evaporation to get groundwater solutions containing 5000 mg /l NH_4-N for treating soil prior to incubation. Well water No. SW 15 (4600 mg /l NH_4-N) did not require concentration.

By concentrating well water No. TW 8 by a factor of 1 : 100 (from 1 litre to 10 ml through a sequence of evaporation), it was expected to get 3975 mg /l NH_4-N and 1025 mg /l NO_3-N in the concentrated well water No. TW 8. However, about half of NH_4-N and NO_3-N of this well water was lost by this method as shown in Table 5.8.

Table 5.8. : Ammonium and nitrate levels in the concentrated well water No. TW 8.

Well No.	NH_4-N (mg /l)	NO_3-N (mg /l)
Concentrated TW 8	2300	775

This loss of ammonium and nitrate may be because ammonium and nitrate were trapped between the crystals of the salts (white precipitate) which were filtered after every time of evaporation. These crystals of salts were present in the iron sludge of this well. The loss of ammonium was not due to the pH because the pH was adjusted to be at pH 5.0 every time before the evaporation.

5.3.3.1.1.2. PREPARATION OF TREATMENT SOLUTION BY CONCENTRATING GROUNDWATER AND ADDITION OF AMMONIUM-N

As a result of the failure of the previous test, it was decided to concentrate the well waters No. TW 8 and SW 11 by a factor of 1 : 20 without filtration and add ammonium sulphate to provide 2500 mg /l NH_4-N. Well water No. SW 15 was diluted to get 2500 mg /l NH_4-N in it. Since the concentration of these well waters was halved to 2500 mg /l NH_4-N double of the volume of each well water will be added to the soil samples.

The concentrated well water No. TW 8 was a suspension with a red-orange colour because there was much of iron oxide in it. The concentrated well water No. SW 11 was a solution with brownish colour.

The ammonium level in the concentrated well waters No. TW 8 and SW 11 and the diluted well water No. SW 15 is given in Table 5.9.

Table 5.9. : Ammonium level in the concentrated well waters No. TW 8 and SW 11 and the diluted well water No. SW 15.

Well No.	Initial NH_4-N (mg /l)	NH_4-N level after treatment (mg /l)	Calculated addition of NH_4-N (mg /l)
Concentrated SW 11	58.2	705	1790
Concentrated TW 8	39.8	437	2060
Diluted SW 15	4600	2510	0.0

0.4868 g of ammonium sulphate (dried at 105 °C for 1 hour) was added to 50 ml of the concentrated well water No. TW 8 and 0.4245 g of ammonium sulphate was

added to 50 ml of the concentrated well water No. SW 11. Then the bottles were shaken well.

The final concentration of ammonium in the concentrated well waters No. TW 8 and SW 11, and the diluted well water No. SW 15 is given in Table 5.10.

Table 5.10. : The final concentration of ammonium in the concentrated well waters No. TW 8 and SW 11, and the diluted well water No. SW 15.

Well No.	NH_4-N (mg /l)
Concentrated SW 11	2490
Concentrated TW 8	2560
Diluted SW 15	2510

5.3.3.2. EFFECT OF GROUNDWATER ON NITRIFICATION IN SOIL

Nitrification rates based on loss of ammonium in the soil samples incubated at -0.5 bar moisture potential and 20 °C calculated from the linear parts of the curves as in section 5.3.2. are given in Table 5.11. Nitrification rates based on formation of nitrate are shown in Table 5.12. The results are the mean of four replicates. The nitrification rates in the control soils (Darvel and Dreghorn) of this experiment are slightly different to those from the preliminary experiment because fresh soil samples were collected for this experiment. As can be seen, ammonium loss and nitrate formation rates were higher in Darvel soil than those of the Dreghorn, Intermediate Oxidation Plant (IOP) and H-Acid soils. Ammonium loss rates showed a decrease in the incubated soils in the following order :

Darvel < Dreghorn < IOP < H-Acid.

Nitrate formation rates showed a decrease in the incubated soils in the following order :

Darvel < Dreghorn < H-Acid < IOP.

In the H-Acid soil, ammonium loss and nitrate formation rates showed a nitrogen imbalance as nitrate formation was more than ammonium loss and this could be attributed to mineralisation taking place.

The Tukey LSD test (p = 0.05) showed that in all soils, ammonium loss and nitrate formation rates in soil treated with well water No. SW 11 were not significantly different (at 5 % level) from those in soil treated with the control treatment. Well water No. SW 11 showed no inhibition in all four soils. Well water No. SW 15 showed significant inhibition in the IOP, Dreghorn and H-Acid soils but not in the Darvel soil. Well water No. TW 8 significantly inhibited all the soils to the extent that nitrification was barely measurable in the Dreghorn, IOP and H-Acid soils. The effect of inhibition was less in the Darvel soil and was greatest in the sandy textured soils (Dreghorn and IOP soils).

Table 5.11. : Nitrification rates based on loss of ammonium in the soil samples incubated at -0.5 bar moisture potential and 20 °C.

Soil sample	Treatment	Ammonium-N loss (mg /kg soil /day) Mean*	95 % C. I.
Darvel soil	2500 mg /l NH_4-N solution	-20.3 a	(-21.8, -18.9)
	Well water No. TW 8	-9.9 c	(-10.5, -9.4)
	Well water No. SW 11	-20.0 a	(-20.8, -19.2)
	Well water No. SW 15	-18.6 b	(-19.6, -17.5)
Dreghorn soil	2500 mg /l NH_4-N solution	-7.7 a	(-8.3, -7.1)
	Well water No. TW 8	0.5 c	(0.4, 0.5)
	Well water No. SW 11	-7.4 a	(-8.0, -6.8)
	Well water No. SW 15	-4.4 b	(-5.1, -3.7)
IOP soil (Soil No. 1)	2500 mg /l NH_4-N solution	-2.9 a	(-3.1, -2.7)
	Well water No. TW 8	0.0 c	(-0.2, 0.3)
	Well water No. SW 11	-2.8 a	(-2.9, -2.6)
	Well water No. SW 15	-0.8 b	(-0.9, -0.7)
H-Acid soil (Soil No. 9)	2500 mg /l NH_4-N solution	-2.3 a	(-2.6, -2.0)
	Well water No. TW 8	1.0 c	(0.8, 1.3)
	Well water No. SW 11	-2.3 a	(-2.4, -2.1)
	Well water No. SW 15	-0.9 b	(-1.0, -0.7)

* For each soil, mean values followed by same letter within a column are not significantly different at 5 % level using Tukey LSD test.

Table 5.12. : Nitrification rates based on formation of nitrate in the soil samples incubated at -0.5 bar moisture potential and 20 °C.

Soil sample	Treatment	Nitrate-N formation (mg /kg soil /day)	
		Mean*	95 % C. I.
Darvel soil	2500 mg /l NH_4-N solution	20.9 a	(20.2, 21.5)
	Well water No. TW 8	11.7 b	(11.1, 12.4)
	Well water No. SW 11	20.6 a	(19.7, 21.4)
	Well water No. SW 15	20.3 a	(16.3, 24.4)
Dreghorn soil	2500 mg /l NH_4-N solution	8.5 a	(8.0, 9.1)
	Well water No. TW 8	0.5 c	(0.5, 0.6)
	Well water No. SW 11	8.4 a	(8.0, 8.8)
	Well water No. SW 15	5.4 b	(5.0, 5.8)
IOP soil (Soil No. 1)	2500 mg /l NH_4-N solution	3.0 a	(2.8, 3.3)
	Well water No. TW 8	0.1 c	(0.1, 0.1)
	Well water No. SW 11	2.9 a	(2.8, 3.0)
	Well water No. SW 15	0.8 b	(0.7, 1.0)
H-Acid soil (Soil No. 9)	2500 mg /l NH_4-N solution	3.7 a	(3.4, 4.0)
	Well water No. TW 8	0.4 c	(0.3, 0.5)
	Well water No. SW 11	3.6 a	(3.4, 3.8)
	Well water No. SW 15	1.9 b	(1.8, 2.0)

* For each soil, mean values followed by same letter within a column are not significantly different at 5 % level using Tukey LSD test.

The inhibitory effect could be due to toxicity or high salinity of the well waters. Well water No. TW 8 had high level of boron (107 mg /l) and well water No. SW 15 had very high total dissolved ions as shown by its high conductivity (EC = 62.0 ms /cm). The inhibition in nitrification caused by well water No. TW 8 could be explained by the presence of boron. Levels of other potentially toxic metals were low (see Table 5.7.). The iron level in well water No. TW 8 is high (95 mg /l) and would be increased by the concentration which was carried out although much was lost from solution by precipitation. These results are consistent with that happened to the grass which was treated with well water No. TW 8 in the first period of treatment of the pot experiment (Chapter 6). Toxicity symptoms appeared on grass just 10 days after the start

of the applications of well water No. TW 8 and were less severe in the H-Acid soil compared to the Intermediate Oxidation Plant (IOP), Safety Fuse and Nylon soils. The inhibition in the Darvel soil is less than that of the other soils and this may be due to the higher clay and oxides contents of the Darvel soil leading to absorption of boron. The very sandy soils such as Dreghorn and IOP soils would have a little absorption capacity for boron, and consequently, greater toxicity and inhibition. Despite the better properties of the H-Acid soil (high clay content, high organic matter and high CEC), nitrification was almost completely inhibited.

Glasscock et al. (1995) concluded that the nitrification inhibitory effects of the two inhibitors, Dicyandiamide (DCD) and N-serve (N-S) in three soils (clay, clay loam and sandy loam) was influenced by soil texture. The influence of high NH_4-N concentrations, nitrification inhibitors and the combinations of the two was strongest on the sandy loam soil. They considered the most likely explanation to be soil texture. The effectiveness of inhibitors increased from the clay soil to the clay loam and to the sandy loam suggesting that absorption of inhibitors influenced their effectiveness. The nitrification inhibition was not related to salt concentrations as measured by electrical conductivity. It seems that in addition to some salt effect, a specific NH_4 concentration effect was observed.

Blaise et al. (1997) found that iron pyrites retarded nitrification of urea-derived ammonium (NH_4), the effect was greatest at the highest level (10000 mg /kg soil). Nitrification inhibition, at the end of 30 days, was 40.3 % with 10000 mg pyrites /kg soil, compared to 55.9 % with dicyandiamide (DCD). The inhibitory effect with lower rates of pyrite (100 - 500 mg /kg) lasted only up to 9 days. The effect of pyrite could be due to the toxic action of any one or a combination of the following : (1) sulphides (Prasad and Reddy, 1977); (2) the oxidised forms of the sulphides, i.e. thiosulphates, tri- and tetrathionates; or (3) presence of Fe^{2+} ions (Goos and Ahrens, 1992).

Mineralization rates in the soil samples incubated at -0.5 bar moisture potential and 20 °C are summarised in Table 5.13. The results are the mean of four replicates. As can be seen, there was negligible mineralization in all treatments of IOP soil. There is little conclusive evidence of inhibition by the well waters.

Table 5.13. : Mineralization rates in the soil samples incubated at -0.5 bar moisture potential and 20 °C.

Soil sample	Treatment	Mineralization rate (mg N /kg soil /day)	
		Mean*	95 % C. I.
Darvel soil	2500 mg /l NH_4-N solution	1.0 b	(0.7, 1.3)
	Well water No. TW 8	1.4 ab	(1.1, 1.6)
	Well water No. SW 11	0.5 c	(0.3, 0.6)
	Well water No. SW 15	1.6 a	(1.0, 2.2)
Dreghorn soil	2500 mg /l NH_4-N solution	0.7 b	(0.4, 0.9)
	Well water No. TW 8	1.0 a	(0.9, 1.1)
	Well water No. SW 11	0.5 bc	(0.4, 0.7)
	Well water No. SW 15	0.4 c	(0.2, 0.5)
IOP soil (Soil No. 1)	2500 mg /l NH_4-N solution	0.0 a	(-0.1, 0.2)
	Well water No. TW 8	0.1 a	(-0.2, 0.3)
	Well water No. SW 11	0.1 a	(-0.0, 0.2)
	Well water No. SW 15	0.1 a	(-0.0, 0.1)
H-Acid soil (Soil No. 9)	2500 mg /l NH_4-N solution	1.1 ab	(1.1, 1.2)
	Well water No. TW 8	1.3 a	(1.1, 1.5)
	Well water No. SW 11	1.0 b	(0.9, 1.1)
	Well water No. SW 15	1.0 b	(1.0, 1.1)

* For each soil, mean values followed by same letter within a column are not significantly different at 5 % level using Tukey LSD test.

CHAPTER 6

FERTILISATION OF GRASS WITH WATER CONTAMINATED WITH AMMONIUM AND NITRATE

6.1. INTRODUCTION

Contaminated groundwater often needs cleaning to prevent contamination of other water resources. The contaminated groundwater can be remediated by different treatments such as physical, chemical and biological treatments. Physical and chemical treatments have disadvantages such as odor, and high chemical cost. However, the advantage of the bioremediation treatment is the relatively low cost.

Bae et al. (1997) emphasised that the methods used for the removal of NH_4-N are physical/chemical or biological processes. However, physical/chemical processes such as stripping, struvite precipitation, and ion exchange have several disadvantages; odor, air pollution, high chemical cost, and excess sludge production (Ehrig, 1989; Bae et al., 1995; and Hwang, 1995).

Trees and ryegrass can be used successfully in the bioremediation treatment of contaminated groundwater. The reason for using the ryegrass not the trees in the present biological process is that (1) the grass has high demand on nitrogen, (2) by cutting the grass frequently the contaminants can be removed.

Reid (1970 and 1972) applied 21 rates of nitrogen fertiliser ranging from 0.0 to 896 kg /ha annually to S. 23 perennial ryegrass swards with and without S. 100 white clover. He reported that the response of dry matter yield to nitrogen rate on the pure-grass sward was almost linear between the 0.0 and 336 kg /ha rates, then it decreased progressively, becoming non-significant about the 560 kg /ha rate. The dry matter yield for pure grass-sward at the maximum fertiliser level (896 kg N /ha) varied from 13.3 to 14.9 t /ha over the six years.

The total nitrogen yield in grass continued to increase even at the highest level of nitrogen applied. The total yield of nitrogen in the harvested leaf tissue for pure grass-sward at the maximum fertiliser level (896 kg N /ha) varied from 486 to 523 kg /ha over the six years (Reid, 1970; and Reid, 1972).

Reid and Strachan (1974) reported that the nitrate-N content of the herbage increased with increasing nitrogen rate from 224 kg /ha upwards, but the potentially toxic level of 0.22 % in dry matter was not reached until the annual nitrogen rate was about 560 kg /ha. On average, at the 896 kg nitrogen /ha rate the non-protein nitrogen content had increased to 27.5 % of the total nitrogen yield, and 40.3 % of the non-protein nitrogen yield consisted of nitrate nitrogen. Nitrate content was shown to be a sensitive indicator of the level of nitrogen nutrition of the herbage, the optimum nitrogen rate for dry matter production coinciding with a nitrate-N content of approximately 1000 mg /kg dry matter. The toxic level of nitrogen is not important in the current study since there is no intention to graze the grass or produce silage.

Reid (1978) pointed out that alterations in the cutting frequency affected the pattern of dry matter yield response to nitrogen, but not that of crude protein yield response. The average total yield of herbage dry matter at 896 kg N /ha was 14.6, 13.9 and 10.5 t /ha for 3 cuts, 5 cuts and 10 cuts, respectively; but the average total yield of nitrogen at 896 kg N /ha was 0.44, 0.47 and 0.45 t /ha for 3 cuts, 5 cuts and 10 cuts, respectively, over the three years.

Griffith et al. (1997b) conducted a study to determine the relationship between plant nitrogen and dry matter accumulation and soil nitrogen status as affected by N-source fertilisation as a function of accumulated growing degree days (GDD). They found that Italian ryegrass (*Lolium multiflorum* Lam. cv. Marshall) grown in western Oregon accumulated the greatest portion of plant nitrogen and dry mass between tiller elongation and mid-heading, and this occurred between approximately 700 to 1100 GDD. In 1992, shoot dry mass accumulation and nitrogen uptake continued over a longer period compared to 1991, but maximum nitrogen accumulation was reached by mid-head emergence (1134 GDD), similar to 1991. In addition, in 1992, plant nitrogen levels declined following mid-head emergence and prior to maximum dry mass accumulation. In 1991, plant nitrogen accumulation preceded dry mass accumulation by approximately 100 GDD; however, in 1992, nitrogen uptake preceded dry matter accumulation by 200 to 300 GDD throughout most of the growing season.

Haycock and Pinay (1993) investigated two sites, grass (*Lolium perenne* L.), and poplar (Populus italica)-vegetated riparian strips, in southern England (River Leach). They pointed out that comparison of the efficiency of the grass and poplar riparian zones suggests that NO_3-N reduction within riparian buffer strips during the

winter months is spatially concentrated at the edge of the zone, possible in as little as the first 5 m of the riparian buffer strip, where groundwater rich in NO_3-N meets a substrate environment that supports denitrifying bacteria. It is postulated that although vegetation has no active role in retaining NO_3-N in the winter, aboveground vegetative biomass does contribute C to the soil microbacterial biomass that is engaged in NO_3-N reduction in the winter months, this accounted for the greater efficiency of the poplar vegetated site.

The irrigation of ryegrass with highly contaminated groundwater could result in problems such as salinity, high or low pH and toxicity. Salinity has an effect on the physiology of plant as it decreases the growth as a result of insufficient water. Heavy metals such as boron, copper, nickel, lead, cadmium and zinc can cause phytotoxicity to ryegrass.

Sagi et al. (1997a) studied the influence of salinity on the activity of nitrate reductase (NR, EC 1.6.6.1) and the level of the molybdenum cofactor (MoCo) as affected by the source and concentration of nitrogen in annual ryegrass (*Lolium multiflorum* cv. Westerwoldicum). They indicated that salinity-treated (11.2 ds /m) plants produced less biomass and more organic nitrogen while accumulating more NO_3-N than control plants. Salinity inhibited shoot growth and increased shoot nitrate reductase activity of plants receiving 4.5 mM NH_4NO_3 or $NaNO_3$. In addition, similar effects were observed in roots of plants grown in 4.5 mM NH_4NO_3.

Fleming (1980) demonstrated that boron toxicity of plants is of particular concern in arid regions where naturally high levels of boron occur. In temperate regions, boron toxicity is most likely to be found on light soils supporting sensitive crops, but its occurrence is usually contingent on fertiliser misuse, e.g., the drilling of boronated compound with a cereal. The sensitivity or tolerance to excess boron depends on rate of boron accumulation rather than to sensitivity of particular plant tissues; for instance, tolerant plants accumulated boron slowly while sensitive plants absorbed rapidly. Boron was concentrated in relatively small necrotic areas near leaf tips or edges, and this localisation is a possible explanation both for the narrow range between deficient and toxic soil solutions, and for the fact that yield decreases associated with boron toxicity are often quite small.

Ylaranta (1996) studied the concentrations of sulphur, zinc, copper, lead and cadmium in spring wheat (*Triticum aestivum*, cv. Ruso) grain and straw, Italian ryegrass (*Lolium multiflorum* L. cv. Amenda), timothy (*Phleum pratense*, cv. Tarmo) and lettuce (*Lactuca sativa*) in a three-year field experiment conducted in southern Finland near a copper-nickel smelter and at nonpolluted control sites. He reported that concentrations of zinc, copper, lead, cadmium and nickel varied between different plant species and also between experimental years. The differences in the sulphur concentrations between plants were smaller than were those in heavy metals. The zinc, copper, lead, nickel and sulphur concentrations in timothy grown at two sites in southern Finland were at the same level but the cadmium concentrations were at a somewhat higher level than the reported average in Finland (Makela-Kurtto et al., 1993). In all cases nickel accumulation by different plants species was intense at Harjavalta. Copper was effectively accumulated by lettuce, timothy and Italian ryegrass, as were zinc and cadmium by plants grown on plots.

Hojito (1998) studied the effect of nitrogen fertiliser application on yield of orchardgrass and soil solution composition of the soil for a Tenpoku acid brown forest soil during a period of 10 years from 1974 to 1985. He summarised that growth decline of grasses in acid soil was as a result of the decrease in phosphorus uptake due to the suppression of root elongation by aluminium. Acid tolerance was correlated with root growth, P uptake, and Al translocation to the shoots. The critical pH of the surface soil for which lime was needed was found to depend on the aluminium tolerance of grass species as follows : orchardgrass, 5.0; timothy and Kentucky bluegrass, 5.1; perennial ryegrass, red clover, and redtop, 5.2; alfalfa and white clover, 5.4.

The bioremediation treatment to clean up the contaminated groundwater is carried out by irrigating ryegrass with the ammonium and nitrate contaminated groundwater. This study was carried out with the following objectives :

1- To determine the influence of ammonium and nitrate contaminated groundwater on the growth of the perennial ryegrass (*Lolium perenne* L.) and soil.
2- To evaluate the efficiency of the perennial ryegrass (*Lolium perenne* L.) in the remediation of high ammonium and nitrate groundwater.

6.2. MATERIALS AND METHODS

OUTLINE OF EXPERIMENT :

Four soil samples [Intermediate Oxidation Plant (IOP) area (soil No. 1), H-Acid area (soil No. 9), Safety Fuse area (soil No. 11) and Nylon area (soil No. 15)] and three well water samples (well waters No. TW 8, SW 11 and SW 15) were used in the pot experiment. Each soil sample was treated with five treatments. Nutrients (nitrogen, phosphorus, potassium and magnesium) were added to the soil as a nutrient solution.

A randomised block design was used in this experiment and was replicated five times.

6.2.1. MATERIALS

6.2.1.1. SOIL SAMPLES

Four soil samples were collected from the Ardeer site for the pot experiment due to their differing texture and organic matter levels. These four soil samples were as follows :

Soil sample from site No. 1 of the IOP area.

Soil sample from site No. 9 of the H-Acid area.

Soil sample from site No. 11 of the Safety Fuse area.

Soil sample from site No. 15 of the Nylon area.

These soil samples are described in full details in section 5.2.1.1.

The four soil samples were collected as described in section 5.2.1.1.1.

6.2.1.1.1. PREPARATION OF THE SOIL SAMPLES FOR THE POT EXPERIMENT

The four soil samples were brought to the laboratory in labelled plastic bags and kept in the cold room at 2 °C until required for analysis and use in the pot experiment.

20 kg of each soil were taken, spread on a clean plastic sheet, the aggregates were broken to get the actual particle size, and each soil sample was mixed thoroughly to minimise the effects of local variations. Then each soil sample was partially air dried at room temperature just sufficiently to allow handling and to pass a 2 mm stainless steel sieve easily. The larger inert material is considered to have little effect on the chemical and nutritional status of the soil. The soil samples were then stored in the cold room at 2 °C until required for the pot experiment.

The physical and chemical analysis of the four soil samples before treatment is described in section 5.2.1.1.3.

6.2.1.2. GROUNDWATER WELLS

6.2.1.2.1. WATER SAMPLES

Three groundwater samples from wells No. TW 8, SW 11 and SW 15, were collected from the Ardeer site. These wells were chosen on the basis of the ammonium-N level and pH as described in section 4.3.1. These wells included wells with a moderate level of ammonium, wells No. SW 11 (Nylon area) and TW 8 (landfill area); and a well with very high level of ammonium, well No. SW 15 (in the middle of the Ardeer site). It was important to choose well water with an approximately neutral pH for this experiment.

The water samples were collected as described in section 4.2.1.1. The locations of the sampling points are given in section 4.2.1.1. and the description of these groundwater wells is given in section 4.2.1.2. The water samples were stored in the cold room at 2 °C until required for analysis and use in the pot experiments.

The chemical analysis of the three groundwater samples before treatment is described in section 5.2.1.2.2.

6.2.1.3. NUTRIENT SOLUTIONS

6.2.1.3.1. NUTRIENT MIXTURE SOLUTION No. 1

This nutrient solution contained a high level of nitrogen because the control treatment needed high nitrogen addition to let the grass to grow well up to the end of the

pot experiment. This nutrient solution contained the following standard levels of nutrients :

100 kg N /ha.

50 kg P /ha.

100 kg K /ha.

50 kg Mg /ha.

The nutrient mixture solutions (No. 1, 2 and 3) contained high levels of phosphorus and potassium as the chemical characteristics of the soil samples (see Table 6.6.) showed a deficiency of phosphorus and potassium in the four soils.

The Safety Fuse soil had low magnesium index (see Table 6.6.), therefore, it was treated with nutrient mixture solution containing nitrogen, phosphorus, potassium and magnesium but the IOP, H-Acid and Nylon soils had level of magnesium, therefore, they were treated with nutrient mixture solution without magnesium.

Nutrient solution No. 2 contained a low level of nitrogen to allow the establishment of the grass before commencing the addition of total nitrogen containing well water.

(a)- PREPARATION OF NUTRIENT MIXTURE SOLUTION No. 1 WITH MAGNESIUM :

37.04 g $(NH_4)_2SO_4$, 19.75 g $NaH_2PO_4.2H_2O$, 15 g KCl and 40.3 g $MgSO_4.7H_2O$ were dissolved with deionised water in 1 litre volumetric flask and the volume was made to the mark with deionised water.

(b)- PREPARATION OF NUTRIENT MIXTURE SOLUTION No. 1 WITHOUT MAGNESIUM :

37.04 g $(NH_4)_2SO_4$, 19.75 g $NaH_2PO_4.2H_2O$ and 15 g KCl were dissolved with deionised water in 1 litre volumetric flask and the volume was made to the mark with deionised water.

6.2.1.3.2. NUTRIENT MIXTURE SOLUTION No. 2

This nutrient solution contained a low level of nitrogen to establish growth of the grass before addition of well waters started. This nutrient solution contained the following levels of nutrients :

20 kg N /ha.

50 kg P /ha*.

100 kg K /ha*.

50 kg Mg /ha*.

* The same levels of nutrients as the standard levels.

(a)- PREPARATION OF NUTRIENT MIXTURE SOLUTION No. 2 WITH MAGNESIUM :

7.41 g $(NH_4)_2SO_4$, 19.75 g $NaH_2PO_4.2H_2O$, 15 g KCl and 40.3 g $MgSO_4.7H_2O$ were dissolved with deionised water in 1 litre volumetric flask and the volume was made to the mark with deionised water.

(b)- PREPARATION OF NUTRIENT MIXTURE SOLUTION No. 2 WITHOUT MAGNESIUM :

7.41g $(NH_4)_2SO_4$, 19.75 g $NaH_2PO_4.2H_2O$ and 15 g KCl were dissolved with deionised water in 1 litre volumetric flask and the volume was made to the mark with deionised water.

6.2.1.3.3. NUTRIENT MIXTURE SOLUTION No. 3

This nutrient solution contained the following standard levels of nutrients with no nitrogen :

50 kg P /ha.

100 kg K /ha.

50 kg Mg /ha.

(a)- PREPARATION OF NUTRIENT MIXTURE SOLUTION No. 3 WITH MAGNESIUM :

19.75 g $NaH_2PO_4.2H_2O$, 15 g KCl and 40.3 g $MgSO_4.7H_2O$ were dissolved with deionised water in 1 litre volumetric flask and the volume was made to the mark with deionised water.

(b)- PREPARATION OF NUTRIENT MIXTURE SOLUTION No. 3 WITHOUT MAGNESIUM :

19.75 g $NaH_2PO_4.2H_2O$ and 15 g KCl were dissolved with deionised water in 1 litre volumetric flask and the volume was made to the mark with deionised water.

6.2.1.3.4. NUTRIENT SOLUTION No. 4

This solution contained 30 kg N /ha and was prepared as follows :

11.11 g $(NH_4)_2SO_4$ was dissolved with deionised water in 1 litre volumetric flask and the volume was made to the mark with deionised water.

CALCULATION FOR THE NUTRIENT SOLUTIONS :

The standard levels of nutrients in the nutrient solution which to be added to the grass were calculated as follows :

$$\text{mg nutrient /pot} = \text{kg nutrient /hectare} \times \frac{\text{Pot area } (m^2)}{10000\ (m^2)} \times 10^6$$

$$\text{mg salt /pot in 10 ml} = \text{mg nutrient /pot} \times \frac{\text{Molecular weight of salt}}{\text{Atomic weight of nutrient} \times \text{Number of atoms}}$$

$$\text{g salt /pot in litre} = \frac{\text{mg salt /pot in 10 ml} \times 100}{1000}$$

Where :

Internal diameter of pot = 10 cm.

Radius (r) = 5 cm.

Area of pot $= \Pi r^2 = 3.14 \times (5^2) = 78.5\ cm^2$

6.2.2. EXPERIMENTAL DESIGN

The experiment was designed to include four soil samples (Intermediate Oxidation Plant (IOP), H-Acid, Safety Fuse and Nylon soils) and three well water samples (well waters No. TW 8, SW 11 and SW 15). Each soil sample was treated with the following treatments :

Control treatment : Standard levels of N, P, K (single treatment).
Treatment No. 1 : Well water No. TW 8 (split treatment).
Treatment No. 2 : Well water No. SW 11 (split treatment).
Treatment No. 3 : Well water No. SW 15 (single treatment).
Treatment No. 4 : Well water No. SW 15 (split treatment).

Nutrients (nitrogen, phosphorus, potassium and magnesium) were added to the soil as a nutrient solution.

A randomised block design was used in this experiment and was replicated five times. The pots were labelled as shown in Table 6.1.

Table 6.1. : The number of each pot.

Soil sample	Treatment	Number
IOP soil (Soil No. 1)	Control treatment	1 - 2 - 3 - 4 - 5
	Treatment No. 1	6 - 7 - 8 - 9 - 10
	Treatment No. 2	11 - 12 - 13 - 14 - 15
	Treatment No. 3	16 - 17 - 18 - 19 - 20
	Treatment No. 4	21 - 22 - 23 - 24 - 25
H-Acid soil (Soil No. 9)	Control treatment	26 - 27 - 28 - 29 - 30
	Treatment No. 1	31 - 32 - 33 - 34 - 35
	Treatment No. 2	36 - 37 - 38 - 39 - 40
	Treatment No. 3	41 - 42 - 43 - 44 - 45
	Treatment No. 4	46 - 47 - 48 - 49 - 50
Safety Fuse soil (Soil No. 11)	Control treatment	51 - 52 - 53 - 54 - 55
	Treatment No. 1	56 - 57 - 58 - 59 - 60
	Treatment No. 2	61 - 62 - 63 - 64 - 65
	Treatment No. 3	66 - 67 - 68 - 69 - 70
	Treatment No. 4	71 - 72 - 73 - 74 - 75
Nylon soil (Soil No. 15)	Control treatment	76 - 77 - 78 - 79 - 80
	Treatment No. 1	81 - 82 - 83 - 84 - 85
	Treatment No. 2	86 - 87 - 88 - 89 - 90
	Treatment No. 3	91 - 92 - 93 - 94 - 95
	Treatment No. 4	96 - 97 - 98 - 99 - 100

6.2.2.1. SET UP OF EXPERIMENT

Because of the variable bulk density of the four soils; 600, 350, 600 and 600 g (on oven dry basis) of the Intermediate Oxidation Plant (IOP), H-Acid, Safety Fuse and Nylon soils, respectively; were weighed into 10 cm internal diameter plastic pots on 14 / 4 / 1998.

The soil surface was wetted with 20 ml of deionised water to provide suitable conditions for germination. The nutrient solutions were added to each pot as follows :

10 ml of nutrient mixture solution No. 1 with magnesium was added by a bulb pipette to the pots of the Safety Fuse soil for the control treatment.

10 ml of nutrient mixture solution No. 1 without magnesium was added by a bulb pipette to the pots of the IOP, H-Acid and Nylon soils for the control treatment.

10 ml of nutrient mixture solution No. 2 with magnesium was added by a bulb pipette to the pots of the Safety Fuse soil for treatments No. 1, 2, 3, and 4.

10 ml of nutrient mixture solution No. 2 without magnesium was added by a bulb pipette to the pots of the IOP, H-Acid and Nylon soils for treatments No. 1, 2, 3, and 4.

This addition of the nutrient solutions was followed by the addition of 20 ml of deionised water to each pot to wash down the nutrient mixture solution.

0.5 g perennial ryegrass seed [*Lolium perenne* L.] was sown in each pot on 15 / 4 / 1998. The pots were covered by black polythene sheet to enhance germination. The germination started on 20 / 4 / 1998.

The pots were transferred to the greenhouse after the completion of germination on 24 / 4 / 1998. The walls of greenhouse were white washed by spraying white paint used by gardeners. The layout plan was as follows :

Layout plan

Block No. 1	: 96-81-61-21-71-31-56-26-41-51-91-1-6-76-86-66-11-46-16-36
Block No. 2	: 57-47-62-92-87-32-7-77-72-2-22-97-27-12-82-42-17-37-67-52
Block No. 3	: 78-28-83-93-8-48-13-33-38-58-18-68-3-73-88-43-53-23-63-98
Block No. 4	: 89-44-94-29-4-9-69-74-84-99-34-24-54-79-14-59-64-39-49-19
Block No. 5	: 25-40-95-90-45-55-65-50-85-5-10-75-70-60-30-80-20-35-15-100

6.2.2.2. STABILITY PERIOD

This period was started on 24 / 4 / 1998. The grass was irrigated with deionised water for 6 weeks throughout this period to establish a good growth of grass for the first and second period of treatment. In the beginning of this period, the grass was irrigated with 25 ml deionised water every time of irrigation, then it needed 50 then

100 then 150 ml of deionised water every time of irrigation due to its water requirement in the later stages of growth.

The irrigation was carried out in the morning or at the end of the day to avoid evaporation if it was carried out at noon.

Yellowing (chlorosis) started to appear in the older leaves of some soil treatments on 13 / 5 / 1998. Therefore, 10 ml of the nutrient solution No. 4 was added to these treatments by a bulb pipette on 16 / 5 / 1998. Then the pots were irrigated with 100 ml deionised water.

The grass was established on 6 / 6 / 1998 and was left without irrigation for three days before cutting to let the soil to be semi fresh and easy for cutting. The grass was cut on 9 / 6 / 1998 with stainless steel scissors at 1 inch above the soil. The grass was discarded.

6.2.2.3. FIRST PERIOD OF TREATMENT

This period was started on 9 / 6 / 1998. The nutrient solutions were added to each pot on 10 / 6 / 1998 as follows :

10 ml of nutrient mixture solution No. 1 with magnesium was added by a bulb pipette to the pots of the Safety Fuse soil for the control treatment.

10 ml of nutrient mixture solution No. 1 without magnesium was added by a bulb pipette to the pots of the Intermediate Oxidation Plant (IOP), H-Acid and Nylon soils for the control treatment.

10 ml of nutrient mixture solution No. 3 with magnesium was added by a bulb pipette to the pots of the Safety Fuse soil for treatments No. 1, 2, 3, and 4.

10 ml of nutrient mixture solution No. 3 without magnesium was added by a bulb pipette to the pots of the IOP, H-Acid and Nylon soils for treatments No. 1, 2, 3, and 4.

The addition of the nutrient mixture solutions was followed by the addition of 25 ml of deionised water to each pot.

CALCULATION FOR THE REQUIRED VOLUME OF THE WELL WATER :

Each treatment of treatments No. 1, 2, 3 and 4 was applied to the grass to provide 100 kg N /ha for each pot. The volume of well water which provides the required level of nitrogen was calculated according to the total nitrogen (mg /kg) in each well water (see Table 6.2.) as follows :

The required volume of well water to provide 100 kg N /ha was calculated from the following equation :

$$\text{Required volume of well water} = \frac{\text{Nitrogen requirement of the pot (78.5 mg N)}}{\text{Total nitrogen (mg /l) of the well water}}$$

Where :

100 kg N /hectare = 1 mg N /cm^2

So :

1 mg N is required per cm^2.

The pot area = 78.5 cm^2.

Therefore :

78.5 mg N is required per pot.

Table 6.2. : Total nitrogen of the groundwater samples.

Well No.	Total nitrogen (mg /l)	Volume required
TW 8	50.0	1.57 litre
SW 11	56.3	1.39 litre
SW 15	10175	7.70 ml

The application of the treatments to the grass was started on 15 / 6 / 1998. The treatments had been applied to the grass throughout this period as shown in Table 6.3.

Table 6.3. : The method of application of the treatments to the grass.

Treatment	Method of application
Control treatment : Standard N, P, K (single treatment).	Standard levels of N, P, K were added for each pot in the first week only. Then 100 ml deionised water was added per pot every time of irrigation for five weeks.
Treatment No. 1 : Well water No. TW 8 (split treatment).	100 ml of well water No. TW 8 was added per pot at every time of irrigation until the required volume (1.57 litre) was added. Then the irrigation was continued with 100 ml deionised water per pot when required until the end of five weeks.
Treatment No. 2 : Well water No. SW 11 (split treatment).	100 ml of well water No. SW 11 was added per pot at every time of irrigation until the required volume (1.39 litre) was added. Then the irrigation was continued with 100 ml deionised water per pot when required until the end of five weeks.
Treatment No. 3 : Well water No. SW 15 (single treatment).	7.7 ml of well water No. SW 15 + 92.3 ml of deionised water were added per pot in the first week only. Then the irrigation was continued with 100 ml deionised water per pot when required until the end of five weeks.
Treatment No. 4 : Well water No. SW 15 (split treatment).	7.7 ml of well water No. SW 15 was added per pot in five weeks as follows : At the start of each week, 1.7, 1.5, 1.5, 1.5 and 1.5 ml of well water No. SW 15 were added in 100 ml deionised water. At all of the times pots were irrigated with 100 ml deionised water as required.

Toxicity symptoms appeared on the grass of the pots treated with well water No. TW 8 on 19 / 6 / 1998. After 24 / 6 / 1998, the irrigation with well water No. TW 8 was stopped, therefore, each pot got only 500 ml of well water No. TW 8. Irrigation was continued for these pots with deionised water from 3 / 7 / 1998 until the end of the five weeks.

The grass was left without irrigation two days before cutting. The grass was cut on 21 / 7 / 1998. See forward (section 6.2.2.5.).

6.2.2.4. SECOND PERIOD OF TREATMENT

This period was started on 21 / 7 / 1998. The nutrient solutions were added to each pot on 22 / 7 / 1998 as described in section 6.2.2.3.

The addition of the nutrient mixture solutions was followed by the irrigation with well water for the pots treated with treatments No. 2, 3 and 4; and with deionised water for the pots treated with the control treatment.

The application of the treatments to the grass was started on 22 / 7 / 1998. The treatments were applied to the grass throughout this period as described in section 6.2.2.3. with the exception that there was no treatment for the pots with treatment No. 1 (well water No. TW 8).

Blackfly and Greenfly started to appear on the grass on 25 / 7 / 1998. The grass was looking good and there was no damage. Rapid solution (primicarb, ICI) was applied at 0.32 % solution on the leaves of the grass on 27 / 7 / 1998. Care was taken to make sure that the solution covered all the leaves of the grass.

The Greenfly and Blackfly had disappeared from the grass on 29 / 7 / 1998 and the grass was growing well.

The grass was left without irrigation two days before cutting. The grass was cut on 9 / 9 / 1998. See forward (section 6.2.2.5.).

6.2.2.5. HARVESTING AND PREPARATION OF GRASS, ROOTS AND SOIL SAMPLES FOR ANALYSIS

At the end of the first and second period of treatment, the fresh grass (shoots) yield was recorded by cutting the grass with stainless steel scissors at 1 inch above the soil with the exception of that at the first period of treatment the shoots of treatment No. 1 which had died were cut at 1 cm on the soil surface to get enough plant material for the chemical analysis since the dead grass was very short. The shoots were wrapped in a 40 cm filter papers, weighed and then dried in the oven at 80 °C for 48 hours. The dry matter yield was recorded by weighing.

At the end of the first period of treatment, the contents of the pots treated with treatment No. 1 were left in the pots without treatment until the end of the second period of treatment.

At the end of the second period of treatment, the soil along with the roots of each pot was partially air dried. Then the roots were picked out by hand and by sieving the soil through a 2 mm stainless steel sieve. The roots were thoroughly washed with tap water followed with deionised water to remove soil particles. The roots were wrapped in a 40 cm filter papers and then dried in the oven at 80 °C for 48 hours. The dry matter yield was recorded by weighing.

Before analysis, the dried shoots and roots were ground by using an electric grinder and passed through a 1.5 mm mesh. Ground samples were sealed in a self adhesive plastic bags and stored until required for analysis.

The air dried soil from each pot was also sealed in plastic bags and stored until analysis.

6.2.2.6. CHEMICAL AND PHYSICAL ANALYSIS OF THE SOIL SAMPLES AFTER EXPERIMENT

Moisture content was determined as described in section 2.7.

pH was measured by a combined glass / reference electrode as in section 2.3.

The electrical conductivity was measured using a Jenway 4070 conductivity meter as described in section 2.5.

Inorganic nitrogen was extracted from the soil samples as described in section 2.17. NH_4-N was determined in the filtrate as described in section 3.1.3.2. using a standard series 0.0 to 1.0 mg /l NH_4-N in 0.5 M K_2SO_4 solution. NO_3-N was determined in the filtrate as described in section 2.11.1.3. using a standard series 0.0 to 2.0 mg /l NO_3-N in 0.5 M K_2SO_4 solution. NO_2-N was determined in the filtrate as described in section 2.11.1.3. using a standard series 0.0 to 1.0 mg /l NO_2-N in 0.5 M K_2SO_4 solution.

6.2.2.7. CHEMICAL ANALYSIS OF SHOOTS AND ROOTS

The shoots and roots samples were digested by the method of Bremner and Mulvaney (1982) as described in section 2.21.

Total N as ammonium-N was measured in the plant digests (shoots and roots) by the method described in section 2.11.1.2. using a standard series 0.0 to 100 mg /l NH_4-N.

6.2.2.8. STATISTICAL ANALYSIS

Analysis of variance was carried out by using Minitab package (Release 9.2).

6.3. RESULTS AND DISCUSSION

6.3.1. CHEMICAL AND PHYSICAL PROPERTIES OF THE SOIL SAMPLES

The textural properties of the soil samples are presented in Table 6.4. The results are the mean of three replicates. As can be seen, the Intermediate Oxidation Plant (IOP), Safety Fuse and Nylon soils were sandy soils, while the H-Acid soil was a clayey soil. Three of the four areas, IOP, H-Acid and Safety Fuse, have imported topsoil. The other area, the Nylon area, is sandy with some development of a natural topsoil.

Table 6.4. : Textural properties of the soil samples.

Soil sample	Coarse + medium sand (%)	Fine sand (%)	Silt (%)	Clay (%)
IOP soil	67.7	16.7	7.8	6.7
H-Acid soil	7.3	7.8	35.2	54.6
Safety Fuse soil	70.2	21.1	4.8	3.1
Nylon soil	71.5	21.2	3.4	2.3

The chemical and physical characteristics of the soil samples before treatment are given in Table 6.5. The results are the mean of three replicates. The pH of the IOP and Nylon soils was approximately neutral, however, the pH of the H-Acid and Safety Fuse soils was slightly acidic. Overall, the electrical conductivity was very low in all four soils. The organic matter content and CEC were higher in the H-Acid soil than the other three soils.

Table 6.5. : Chemical and physical characteristics of the soil samples before treatment.

Soil sample	pH	EC* (ms /cm)	Organic matter (%)	CEC (c $mole_c$ /kg)
IOP soil	6.8	0.21	4.4	14.3
H-Acid soil	6.0	0.14	18.2	59
Safety Fuse soil	5.9	0.27	2.9	8.7
Nylon soil	6.9	0.09	1.9	7.1

* EC = Electrical conductivity.

The extractable nutrients in the soil samples before treatment are illustrated in Table 6.6. The results are the mean of three replicates. The nitrate level was slightly higher in the H-Acid soil than the other three soils. Ammonium, nitrite and potassium levels were low in all four soils. Phosphorus level was low in the IOP, H-Acid and Safety Fuse soils, while the Nylon soil had slightly higher extractable phosphorus. The magnesium level was very high in the H-Acid soil, and satisfactory in the IOP and Nylon soils, but low in the Safety Fuse soil. Therefore, the Safety Fuse soil was treated with a background nutrient mixture solution containing nitrogen, phosphorus, potassium and magnesium; but the IOP, H-Acid and Nylon soils were treated with nutrient mixture solution without magnesium.

Table 6.6. : The extractable nutrients in the soil samples before treatment.

Soil sample	NH_4-N (mg /kg)	NO_3-N (mg /kg)	NO_2-N (mg /kg)	P (mg /kg)	K (mg /kg)	Mg (mg /kg)
IOP soil	1.0	1.3	0.2	7.6	52	73
H-Acid soil	2.2	27.2	0.2	10.4	47.3	472
Safety Fuse soil	0.6	0.1	0.3	6.2	23.3	20.1
Nylon soil	0.8	6.8	0.2	44.5	31.1	61

6.3.2. WATER QUALITY PARAMETERS

The chemical composition of the groundwater samples is summarised in Table 6.7. The metal content of the groundwater samples is shown in Table 6.8. As can be seen, the pH was slightly high in all three wells. The well waters varied in the nitrogen, electrical conductivity and composition of the dissolved salts. The heavy metals were low in all well waters.

In terms of fertilising, the levels of nitrogen in the well waters determined the irrigation volume. Well waters No. TW 8 and SW 11 had moderate nitrogen contents, therefore, they were added at a high volume (100 ml per application three times a week until the required volume 1.57 litre of well water No. TW 8 or 1.39 litre of well water No. SW 11 was added). However, well water No. SW 15 had a very high nitrogen content, therefore it was added at a low volume [7.7 ml of well water No. SW 15 diluted in 100 ml was added in the beginning of the growth period in the case of the single treatment and 1.7, 1.5, 1.5, 1.5 and 1.5 ml of well water No. SW 15 diluted in 100 ml was added at the start of each week in case of the split treatment]. Relative to the nitrogen content in well waters No. SW 15 and TW 8, the electrical conductivity was high in these wells, thus saline well waters were added but much diluted in the case of well water No. SW 15.

Table 6.7. : Chemical composition of the groundwater samples.

Well No.	pH	EC* (ms /cm)	NH_4-N (mg /l)	NO_3-N (mg /l)	NO_2-N (mg /l)	Total N (mg /l)
TW 8	7.3	4.7	39.8	9.9	0.4	50.0
SW 11	8.0	0.7	51	5.0	< 0.1	56
SW 15	7.5	62	4600	5580	0.1	10175

Well No.	Cl (mg /l)	K (mg /l)	Na (mg /l)	Ca (mg /l)	Mg (mg /l)	Fe (mg /l)
TW 8	1170	53.8	126	520	297	95
SW 11	24.1	5.0	25.8	55	5.7	9.4
SW 15	343	350	1610	1380	1430	9.2

* EC = Electrical conductivity.

Table 6.8. : Metals content of the groundwater samples determined by the Inductively Coupled Plasma (ICP) Spectroscopy.

Well No.	Cu (mg /l)	Cd (mg /l)	Cr (mg /l)	Zn (mg /l)	Ni (mg /l)	Pb (mg /l)
TW 8	< 0.01	< 0.01	< 0.01	0.3	< 0.01	< 0.1
SW 11	< 0.01	< 0.01	< 0.01	0.4	< 0.01	< 0.1
SW 15	< 0.01	< 0.01	< 0.01	0.6	0.21	< 0.1

Well No.	B (mg /l)	Ba (mg /l)	Mn (mg /l)	K (mg /l)
TW 8	107	0.04	1.0	50.7
SW 11	0.82	0.30	0.3	5.9
SW 15	1.12	0.08	7.9	324

6.3.3. POT EXPERIMENT

The pot experiment was conducted to determine the influence of ammonium and nitrate contaminated groundwater on the growth of the perennial ryegrass (*Lolium perenne* L.) and soil. In addition, the experiment was carried out to evaluate the efficiency of the perennial ryegrass (*Lolium perenne* L.) in the remediation of high ammonium and nitrate contaminated groundwater under controlled conditions in the greenhouse.

The fresh yield of the shoots is shown in Table 6.9. The results are the mean of five replicates. The overall view of the pot experiment in the first period of treatment is presented in Figure 6.1. The comparison of the grass treated with the well water treatments and the control treatment in the first period of treatment is illustrated in Figure 6.2.

Table 6.9. : The fresh yield of the shoots.

Soil and treatment	Fresh yield of shoots [First period] (g /pot)	Fresh yield of shoots [Second period] (g /pot)
IOP soil (Soil No. 1) :		
Standard N, P, K control (single)	13.75	17.14
Well water No. TW 8 (split)	1.70	-----
Well water No. SW 11 (split)	11.84	16.26
Well water No. SW 15 (single)	13.02	16.95
Well water No. SW 15 (split)	12.09	17.73
H-Acid soil (Soil No. 9) :		
Standard N, P, K control (single)	14.84	20.25
Well water No. TW 8 (split)	2.97	-----
Well water No. SW 11 (split)	13.37	18.11
Well water No. SW 15 (single)	14.82	17.90
Well water No. SW 15 (split)	12.43	18.34
Safety Fuse soil (Soil No. 11) :		
Standard N, P, K control (single)	13.72	16.30
Well water No. TW 8 (split)	1.35	-----
Well water No. SW 11 (split)	12.32	17.50
Well water No. SW 15 (single)	13.09	15.48
Well water No. SW 15 (split)	11.30	15.76
Nylon soil (Soil No. 15) :		
Standard N, P, K control (single)	14.34	18.73
Well water No. TW 8 (split)	1.76	-----
Well water No. SW 11 (split)	11.75	17.51
Well water No. SW 15 (single)	13.64	17.84
Well water No. SW 15 (split)	11.64	16.47

----- = No yield.

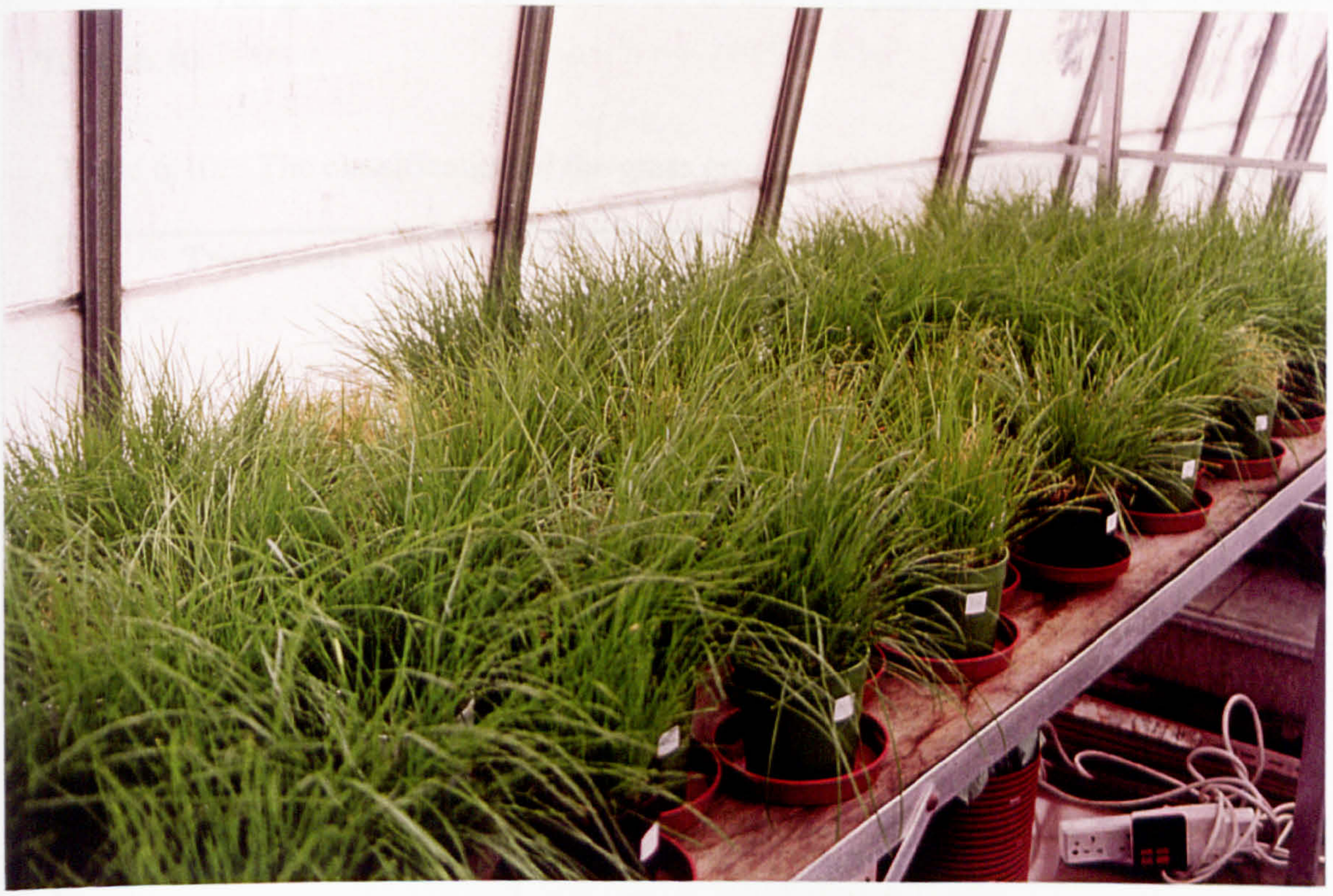


Figure 6.1. : The overall view of the pot experiment in the first period of treatment.

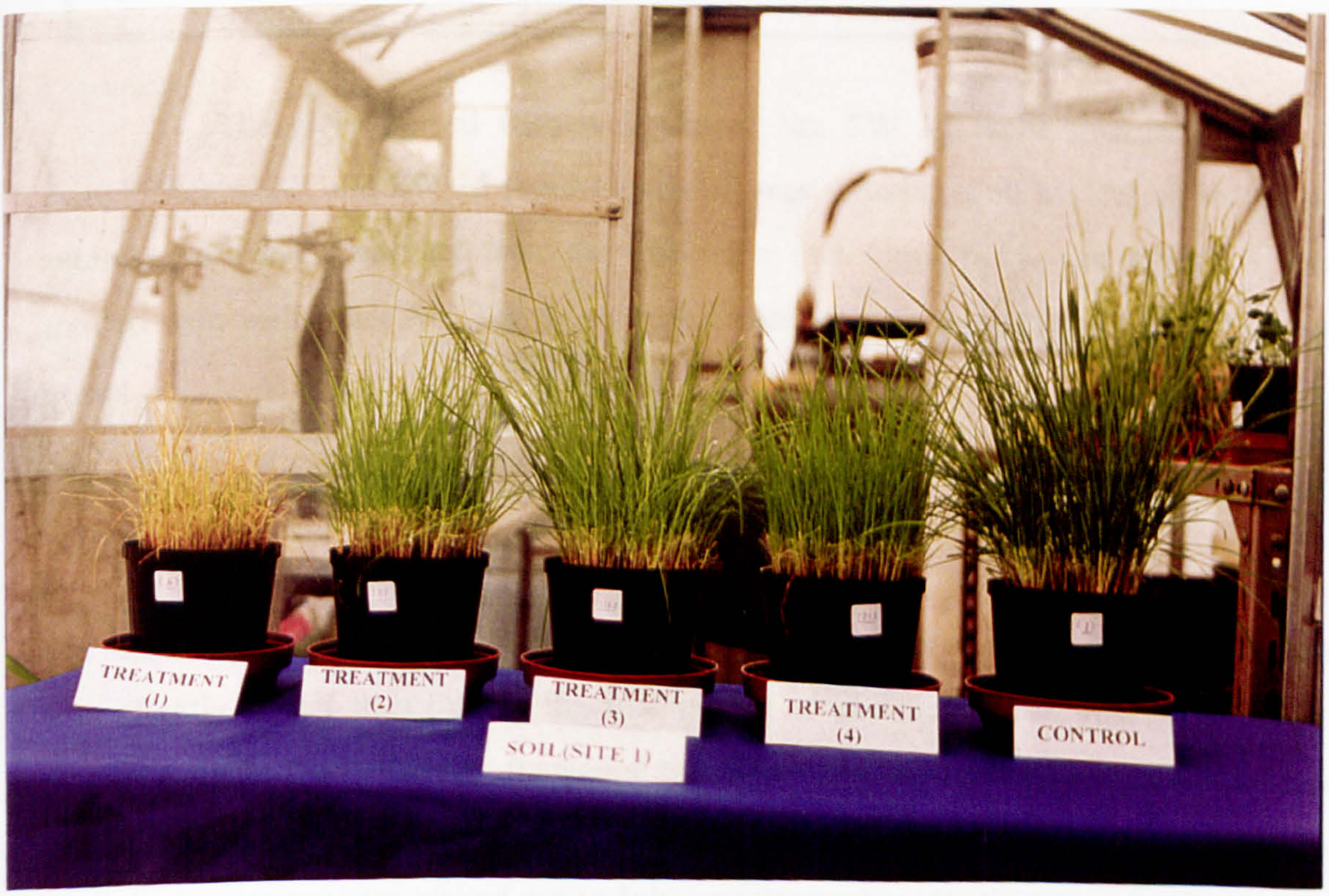


Figure 6.2. : The comparison of the grass treated with the well water treatments and the control treatment in the first period of treatment.

The grass growth was classified in the first period of treatment as shown in Table 6.10.

Table 6.10. : The classification of the grass growth in the first period of treatment.

Treatment	Grass		
	Stem	Leaf	Growth
Standard N, P, K control (single treatment).	Tall.	Tall, thick and dark green.	Good.
Well water No. TW 8 (split treatment).	Short.	Short, thin and green-yellow.	Poor.
Well water No. SW 11 (split treatment).	Tall.	Tall, thick and dark green.	Good.
Well water No. SW 15 (single treatment).	Tall.	Tall, thick and dark green.	Good.
Well water No. SW 15 (split treatment).	Tall.	Tall, thick and dark green.	Good.

The grass treated with well waters No. SW 11 (split treatment), SW 15 (single treatment) and SW 15 (split treatment) was growing well indicating that it was taking up sufficient nitrogen from the well water (see figure 6.2.). The growth of grass treated with well water No. TW 8 (split treatment) was very poor suggesting a toxicity effect.

The dry yield of the shoots and roots is given in Table 6.11. The results are the mean of five replicates. The dry yield of the shoots of the second period of treatment was slightly higher than that of the first period of treatment in all four soils. This is possibly due to the day length (sunshine hours) and the intensity of sunshine; but since the first period of treatment was carried out in June and July, 1998 and the second period of treatment was carried out in July and September, 1998 it is more likely due to the residual effects of nitrogen of the previous treatments.

The residual effect could be due to nitrogen mobilisation from the roots and stubble. Wilkins et al. (1997) assessed the varietal differences in rates of NO_3-N uptake and remobilisation of nitrogen (N) during a cycle of severe defoliation and regrowth in perennial ryegrass (*Lolium perenne* L.) varieties Ba11778, Aberelan, Talbot and Gator. They demonstrated that more N was remobilised from the roots than from the stubble after defoliation. However, there were no significant differences among varieties in the amount and rate of N remobilisation following defoliation.

Regarding the dry yield of the shoots of the first and second period; in all four soils, the dry yield of grass treated with well water No. SW 15 (single treatment) and the control treatment (single treatment) was more than that of grass treated with the other treatments. This is attributed to the fact that the nitrogen fertiliser was added at the start of the growth period, and consequently being used more efficiently to produce dry matter yield.

The grass treated with well water No. TW 8 had the lowest dry yield in all four soils in the first period of treatment and it did not grow in the second period of treatment. Among the well water treatments, well water No. SW 15 (single treatment) was superior in producing dry yield in all four soils. In the five treatments, the dry yield of grass grown in the H-Acid soil was slightly higher than that grown in the other three soils.

In the case of the shoots of the first period, the Tukey LSD test ($p = 0.05$) showed that in all four soils, the dry yield of grass treated with well water No. TW 8 was significantly different from that of grass treated with the other well water treatments. In all four soils, the dry yield of grass treated with well water No. SW 11 was not significantly different (at 5 % level) from that of grass treated with well water No. SW 15 (split treatment), while the dry yield of grass treated with well water No. SW 15 (single treatment) was significantly different from that of grass treated with well water No. SW 15 (split treatment). In the IOP, Safety Fuse and Nylon soils, the dry yield of grass treated with the well water treatments was significantly different from that of grass treated with the control treatment. In the H-Acid soil, the dry yield of grass treated with well waters No. TW 8, SW 11 and SW 15 (split treatment) was significantly different from that of grass treated with the control treatment, while the dry yield of grass treated

with well water No. SW 15 (single treatment) was not significantly different from that of grass treated with the control treatment.

In the case of the shoots of the second period; the Tukey LSD test (p = 0.05) showed that in the IOP and Nylon soils, the dry yield of grass treated with well waters No. SW 11 and SW 15 (split treatment) was significantly different (at 5 % level) from that of grass treated with the control treatment, while the dry yield of grass treated with well water No. SW 15 (single treatment) was not significantly different from that of grass treated with the control treatment. In the H-Acid soil, the dry yield of grass treated with the well water treatments was significantly different from that of grass treated with the control treatment. In the Safety Fuse soil, the dry yield of grass treated with the well water treatments was not significantly different from that of grass treated with the control treatment. The dry yield of grass treated with well water No. SW 15 (single treatment) was not significantly different from that of grass treated with well water No. SW 15 (split treatment) in the H-Acid and Safety Fuse soils, and was significantly different from that of grass treated with well water No. SW 15 (split treatment) in the IOP and Nylon soils.

The dry yield of the roots was higher than that of the shoots of the first or second period of treatment.

Regarding the dry yield of the roots, the Tukey LSD test (p = 0.05) showed that in the IOP, Safety Fuse and Nylon soils; the dry yield of grass treated with well waters No. SW 11, SW 15 (single treatment) and SW 15 (split treatment) was not significantly different (at 5 % level) from that of grass treated with the control treatment, while the dry yield of grass treated with well water No. TW 8 was significantly different from that of grass treated with the control treatment and the other well water treatments. In the H-Acid soil, the dry yield of grass treated with well waters No. TW 8, SW 11 and SW 15 (split treatment) was significantly different from that of grass treated with the control treatment; while the dry yield of grass treated with well water No. SW 15 (single treatment) was not significantly different from that of grass treated with the control treatment. The dry yield of grass treated with well water No. SW 15 (single treatment) was not significantly different from that of grass treated with well water No. SW 15 (split treatment) in the IOP, Safety Fuse and Nylon soils, but was significantly different

from that of grass treated with well water No. SW 15 (split treatment) in the H-Acid soil.

Table 6.11. : The dry yield of the shoots and roots.

Soil and treatment	Dry yield of shoots [First period] (g /pot)*	Dry yield of shoots [Second period] (g /pot)*	Dry yield of roots (g /pot)*
IOP soil (Soil No. 1) :			
Standard N, P, K control (single)	3.43 a	4.42 a	13.36 a
Well water No. TW 8 (split)	0.59 d	-----	3.47 b
Well water No. SW 11 (split)	2.40 c	3.27 b	9.77 a
Well water No. SW 15 (single)	2.98 b	4.29 a	10.48 a
Well water No. SW 15 (split)	2.33 c	3.90 ab	9.50 a
H-Acid soil (Soil No. 9) :			
Standard N, P, K control (single)	3.61 a	5.42 a	8.37 ab
Well water No. TW 8 (split)	1.12 c	-----	3.28 c
Well water No. SW 11 (split)	2.40 b	3.34 c	6.09 bc
Well water No. SW 15 (single)	3.18 a	4.41 b	7.56 ab
Well water No. SW 15 (split)	2.47 b	4.15 b	10.19 a
Safety Fuse soil (Soil No. 11) :			
Standard N, P, K control (single)	3.36 a	3.61 a	9.32 a
Well water No. TW 8 (split)	0.50 d	-----	2.87 b
Well water No. SW 11 (split)	2.22 c	3.29 a	9.75 a
Well water No. SW 15 (single)	2.80 b	3.71 a	11.38 a
Well water No. SW 15 (split)	2.13 c	3.52 a	12.14 a
Nylon soil (Soil No. 15) :			
Standard N, P, K control (single)	3.38 a	4.87 a	12.35 a
Well water No. TW 8 (split)	0.64 d	-----	3.54 b
Well water No. SW 11 (split)	2.13 c	3.51 b	10.33 a
Well water No. SW 15 (single)	2.74 b	4.45 a	9.07 a
Well water No. SW 15 (split)	2.16 c	3.87 b	13.61 a

----- = No yield.

* For each soil, mean values followed by same letter within a column are not significantly different at 5 % level using Tukey LSD test.

Well water No. TW 8 had a clear negative effect on the growth of grass as the yield of this treatment was the lowest in all four soils in the first period and there was no growth in the second period. Toxicity symptoms appeared on the grass of the pots treated with this well water (see Figures 6.3. and 6.4.). Toxicity symptoms appeared on the grass 19 / 6 / 1998 just 10 days after the start of the applications of well water No. TW 8.

These toxicity symptoms were as follows :

- The grass had poor growth and was clearly dying.
- The leaves were short and had light green or green yellow colour (chlorosis) with spotting of brown colour (dead leaf tissue) at the tips and the edges and toward the centre between the veins.

There was also an iron precipitate (orange colour) on the soil surface.

These symptoms were toxicity symptoms not deficiency symptoms because the grass for each soil treated with the control treatment was growing well indicating no micro or macro nutrients deficiency. These toxicity symptoms were not related to aluminium or manganese toxicity because the pH of well water No. TW 8 was not acidic. These toxicity symptoms seemed to be related to boron because further analysis of composition of well water No. TW 8 showed high level of boron (107 mg /l). Alternatively, it may have been related to salinity effect as well water No. TW 8 had high electrical conductivity (4.7 ms /cm).

Ayers and Westcot (1985) reported that boron toxicity symptoms normally show first on older leaves as a yellowing, spotting, or drying of leaf tissue at the tips and edges. As more and more boron accumulates with time, drying and chlorosis often progress toward the centre between the veins (interveinal). In addition, on seriously affected trees such as almonds and other tree crops which do not show typical leaf symptoms, a gum or exudate on limbs or trunk is often noticeable.

These symptoms match the symptoms appeared on the grass treated with well water No. TW 8.

Fleming (1980) reported that when the boron concentrations associated with different degrees of injury were measured, necrotic spots usually contained over 1500 mg /l B while chlorotic tissue levels were mostly between 1000 and 1500 mg /l B.

Richards (1954) pointed out that boron is very toxic to certain plant species, however, the concentration that will injure these sensitive plants is often approximately that required for normal growth of very tolerant plants. For instance, lemons show definite and, at times, economically important injury when irrigated with water containing 1.0 mg /l of boron, while alfalfa will make maximum growth with 1.0 to 2.0 mg /l of boron.

Ayers and Westcot (1985) reported that boron is needed in relatively small amounts, however, it becomes toxic if present in amounts appreciably greater than needed. For some crops, if 0.2 mg /l boron in water is essential, 1.0 to 2.0 mg /l may be toxic. As the boron concentrations in leaf blades exceed 250 - 300 mg /kg (dry weight), most crop toxicity symptoms result, however, not all sensitive crops accumulate boron in leaf blades.

The toxicity effects were severe in the IOP, Safety Fuse and Nylon soils but were less so in the H-Acid soil. On 20 / 7 / 1998, 42 days after the start of the applications of well water No. TW 8, the grass of the H-Acid soil had about 40 % live buds and about 60 % dead grass but the grass of the IOP, Safety Fuse and Nylon soils had about 10 % live buds and about 90 % dead grass. The H-Acid soil was resisting the toxicity of boron possibly because of its high clay content and high organic matter content.

Fleming (1980) showed that the great availability of boron in surface soils compared with subsurface soils, is undoubtedly related to relative organic matter levels and in humid regions, where any soluble salts are readily leached out, available boron is largely held in the organic fraction. Light-textured soils contain less available boron than heavy textured soils and boron deficiency is more common in them because of less binding and therefore greater leaching. In alkaline soils where free calcium ions are present, boron availability is much less; therefore, under these conditions more boron is required to prevent deficiency while on the other hand plants can tolerate amounts of boron that would normally prove toxic.

Figure 6.3. : The comparison of the grass treated with well water No. TW 8 and the control treatment in the first period of treatment.

Figure 6.4. : Close up of the toxicity symptoms in grass treated with well water No. TW 8 in the first period of treatment.

The nitrogen (%) in the shoots and roots is summarised in Table 6.12. The results are the mean of five replicates. As can be seen, the nitrogen (%) of the shoots was slightly higher than that of roots.

Regarding the shoots of the first period, the results for well water No. TW 8 were influenced by the boron toxicity, and the application of this treatment was stopped and the grass died. Therefore, the nitrogen results were not meaningful. Well water No. SW 15 (split treatment) gave higher nitrogen (%) but lower dry yield than well water No. SW 15 (single treatment) and this is attributed to that well water No. SW 15 (split treatment) had nitrogen late at the end of the first period but was unable to make use of it.

The Tukey LSD test ($p = 0.05$) showed that in all four soils, the nitrogen (%) in grass treated with well waters No. TW 8, SW 11 and SW 15 (split treatment) was significantly different (at 5 % level) from that in grass treated with the control treatment. The nitrogen (%) in grass treated with well water No. SW 15 (single treatment) was not significantly different from that in grass treated with the control treatment in the IOP, Safety Fuse and Nylon soils; and was significantly different from that in grass treated with the control treatment in the H-Acid soil. In all four soils, the nitrogen (%) in grass treated with well water No. SW 15 (single treatment) was significantly different from that in grass treated with well water No. SW 15 (split treatment).

Regarding the shoots of the second period, the shoots of grass treated with well water No. SW 11 had higher nitrogen (%) than that treated with the other treatments in all four soils and this could be because the grass treated with well water No. SW 11 had dry yield slightly lower than that treated with the other treatments.

The Tukey LSD test ($p = 0.05$) showed that the nitrogen (%) in grass treated with well water No. SW 11 was significantly different (at 5 % level) from that in grass treated with the control treatment in the IOP, H-Acid and Nylon soils; but not significantly different from that in grass treated with the control treatment in the Safety Fuse soil. The nitrogen (%) in grass treated with well water No. SW 15 (single treatment) was significantly different from that in grass treated with the control treatment in the IOP and Safety Fuse soils; and not significantly different from that in grass treated with the control treatment in the H-Acid and Nylon soils. The nitrogen (%)

in grass treated with well water No. SW 15 (split treatment) was not significantly different from that in grass treated with the control treatment in the H-Acid soil; while it was significantly different from that in grass treated with the control treatment in the IOP, Safety Fuse and Nylon soils. The nitrogen (%) in grass treated with well water No. SW 15 (single treatment) was significantly different from that in grass treated with well water No. SW 15 (split treatment) in the IOP, Safety Fuse and Nylon soils; and not significantly different from that in grass treated with well water No. SW 15 (split treatment) in the H-Acid soil.

Regarding the roots, the nitrogen (%) in the roots was slightly lower than that of the shoots. In the five treatments, the nitrogen (%) in the roots of grass grown in the H-Acid soil was slightly higher than that of grass grown in the other three soils.

The Tukey LSD test ($p = 0.05$) showed that the nitrogen (%) in grass treated with the well water treatments was significantly different (at 5 % level) from that in grass treated with the control treatment in the IOP soil, and not significantly different from that in grass treated with the control treatment in the Safety Fuse soil. In the H-Acid soil, the nitrogen (%) in grass treated with well waters No. TW 8, SW 15 (single treatment) and SW 15 (split treatment) was significantly different from that in grass treated with the control treatment, while the nitrogen (%) in grass treated with well water No. SW 11 was not significantly different from that in grass treated with the control treatment. In the Nylon soil, the nitrogen (%) in grass treated with well waters No. TW 8 and SW 15 (single treatment) was not significantly different from that in grass treated with the control treatment, but the nitrogen (%) in grass treated with well waters No. SW 11 and SW 15 (split treatment) was significantly different from that in grass treated with the control treatment. The nitrogen (%) in grass treated with well water No. SW 15 (single treatment) was not significantly different from that in grass treated with well water No. SW 15 (split treatment) in the IOP, H-Acid and Safety Fuse soils, however, it was significantly different from that in grass treated with well water No. SW 15 (split treatment) in the Nylon soil.

Table 6.12. : Nitrogen (%) in the shoots and roots.

Soil and treatment	N (%) of shoots [First period]*	N (%) of shoots [Second period]*	N (%) of roots*
IOP soil (Soil No. 1) :			
Standard N, P, K control (single)	1.07 c	1.00 bc	0.53 b
Well water No. TW 8 (split)	2.25 a	-----	0.62 ab
Well water No. SW 11 (split)	1.43 b	1.35 a	0.74 a
Well water No. SW 15 (single)	1.05 c	0.88 c	0.65 ab
Well water No. SW 15 (split)	1.57 b	1.10 b	0.65 ab
H-Acid soil (Soil No. 9) :			
Standard N, P, K control (single)	1.21 c	0.99 b	0.90 a
Well water No. TW 8 (split)	2.52 a	-----	0.84 ab
Well water No. SW 11 (split)	1.47 b	1.31 a	0.90 a
Well water No. SW 15 (single)	1.04 d	0.96 b	0.78 b
Well water No. SW 15 (split)	1.40 b	1.09 b	0.80 b
Safety Fuse soil (Soil No. 11) :			
Standard N, P, K control (single)	0.94 d	1.21 a	0.73 a
Well water No. TW 8 (split)	2.23 a	-----	0.63 a
Well water No. SW 11 (split)	1.37 c	1.30 a	0.61 a
Well water No. SW 15 (single)	1.03 d	0.88 b	0.56 a
Well water No. SW 15 (split)	1.54 b	1.13 ab	0.54 a
Nylon soil (Soil No. 15) :			
Standard N, P, K control (single)	1.14 c	0.98 c	0.56 ab
Well water No. TW 8 (split)	2.40 a	-----	0.65 ab
Well water No. SW 11 (split)	1.59 b	1.28 a	0.72 a
Well water No. SW 15 (single)	1.28 c	0.93 c	0.64 ab
Well water No. SW 15 (split)	1.63 b	1.12 b	0.53 b

----- = No yield.

* For each soil, mean values followed by same letter within a column are not significantly different at 5 % level using Tukey LSD test.

The nitrogen yield in the shoots and roots and the apparent nitrogen (%) recovered in the shoots are presented in Table 6.13. The results are the mean of five replicates. It was observed that the nitrogen yield in the shoots of the second period was higher than that of the first period. The nitrogen yield in the roots was higher than that in the shoots of the first or second period of treatment.

In the case of the shoots of the first and second period, the nitrogen yield in grass treated with the well water treatments was lower than that in grass treated with the control treatment in all soils except the Safety Fuse soil. The nitrogen yield in grass treated with well water No. SW 15 (split treatment) was higher than that in grass treated with well water No. SW 15 (single treatment) in all four soils. The nitrogen yield in grass grown in the H-Acid soil was higher than that in grass grown in the other three soils. Among the four soils, the nitrogen yield in grass grown in the Safety Fuse soil was the lowest in the five treatments.

In all soils except the IOP soil, well water No. SW 15 (split treatment) produced higher nitrogen yield in the roots than well water No. SW 15 (single treatment). The nitrogen yield in the roots of grass grown in the H-Acid soil was higher than that of grass grown in the other three soils.

The total nitrogen yield in the shoots was calculated by adding together the nitrogen yield of shoots of both the first and second period of treatment. The grass treated with well water No. SW 15 (split treatment) and the control treatment was more efficient in nitrogen uptake than that treated with well water No. SW 15 (single treatment) in all four soils. The total nitrogen yield in the shoots of grass grown in the H-Acid soil was higher than that of grass grown in the other three soils, however, the total nitrogen yield in the shoots of grass grown in the Safety Fuse soil was lower than that of grass grown in the other three soils.

The Tukey LSD test ($p = 0.05$) showed that the total nitrogen yield in the shoots of grass treated with the well water treatments was significantly different (at 5 % level) from that of grass treated with the control treatment in the H-Acid and Nylon soils. In the IOP and Safety Fuse soils, the total nitrogen yield in the shoots of grass treated with well waters No. SW 11 and SW 15 (split treatment) was not significantly

different from that of grass treated with the control treatment, while the total nitrogen yield in the shoots of grass treated with well water No. SW 15 (single treatment) was significantly different from that of grass treated with the control treatment. The total nitrogen yield in the shoots of grass treated with well water No. SW 15 (single treatment) was significantly different from that of grass treated with well water No. SW 15 (split treatment) in the IOP and Safety Fuse soils, but not significantly different from that of grass treated with well water No. SW 15 (split treatment) in the H-Acid and Nylon soils.

The apparent nitrogen (%) recovered in the shoots was calculated from the total nitrogen yield of the shoots. The apparent nitrogen (%) recovered in the shoots of grass treated with well water No. TW 8 was not calculated because the grass died. The grass treated with well water No. SW 15 (single treatment) had the lowest efficiency in nitrogen uptake in all four soils. The apparent nitrogen (%) recovered in the shoots of grass grown in the H-Acid soil was higher than that of grass grown in the other three soils, however, the apparent nitrogen (%) recovered in the shoots of grass grown in the Safety Fuse soil was lower than that of grass grown in the other three soils.

Table 6.13. : The nitrogen yield in the shoots and roots and the apparent nitrogen (%) recovered in the shoots.

Soil and treatment	N yield of shoots [First Period] (mg /pot)	N yield of shoots [Second Period] (mg /pot)	N yield of roots (mg/pot)	Total N yield of shoots* (mg/pot)	Apparent nitrogen (%) recovered in shoots
IOP soil (Soil No. 1) :					
Standard N, P, K control(single)	36.84	44.05	68.74	80.89 a	51.5
Well water No. TW 8 (split)	13.22	-----	21.18	13.22	-----
Well water No. SW 11 (split)	34.28	43.85	68.80	78.14 a	49.8
Well water No. SW 15 (single)	31.32	37.26	66.62	68.57 b	43.7
Well water No. SW 15 (split)	36.36	42.59	60.76	78.94 a	50.3
H-Acid soil (Soil No. 9) :					
Standard N, P, K control(single)	43.59	53.60	75.10	97.19 a	61.9
Well water No. TW 8 (split)	28.25	-----	27.25	28.25	-----
Well water No. SW 11 (split)	35.27	43.61	54.12	78.88 b	50.2
Well water No. SW 15 (single)	32.85	41.57	58.81	74.42 b	47.4
Well water No. SW 15 (split)	34.58	45.08	81.69	79.66 b	50.7
Safety Fuse soil (Soil No. 11) :					
Standard N, P, K control(single)	31.59	42.00	64.58	73.59 a	46.9
Well water No. TW 8 (split)	11.09	-----	18.18	11.09	-----
Well water No. SW 11 (split)	30.45	42.73	56.90	73.18 a	46.6
Well water No. SW 15 (single)	28.75	32.49	59.64	61.24 b	39.0
Well water No. SW 15 (split)	32.69	39.55	64.07	72.24 a	46.0
Nylon soil (Soil No. 15) :					
Standard N, P, K control(single)	38.70	47.49	67.06	86.20 a	54.9
Well water No. TW 8 (split)	15.15	-----	23.12	15.15	-----
Well water No. SW 11 (split)	33.90	45.08	74.09	78.98 b	50.3
Well water No. SW 15 (single)	35.07	41.32	57.53	76.39 b	48.7
Well water No. SW 15 (split)	35.12	43.29	67.43	78.41 b	49.9

----- = No yield.

* For each soil, mean values followed by same letter within a column are not significantly different at 5 % level using Tukey *LSD* test. Treatment with well water No. TW 8 was not included in the statistical analysis.

The residual nitrogen and nitrogen (%) remaining in the soil after treatment are shown in Table 6.14. The results are the mean of five replicates. As can be seen, ammonium and nitrate were the dominant ions remaining in the soil at the end of the pot experiment, but ammonium level was higher than the nitrate and nitrite levels in all four soils.

The grass treated with well water No. TW 8 resulted in higher residual nitrogen in all four soils than that treated with the other four treatments. The grass treated with well water No. SW 15 (single treatment) resulted in slightly higher residual nitrogen in all four soils than that treated with well water No. SW 15 (split treatment). The grass treated with the control treatment gave the lowest residual nitrogen in all soils except the Safety Fuse soil. The residual nitrogen in the H-Acid soil was higher than in the other three soils.

The Tukey LSD test ($p = 0.05$) showed that the residual nitrogen in the IOP, H-Acid and Safety Fuse soils with the well water treatments was not significantly different (at 5 % level) from that with the control treatment. The residual nitrogen in the Nylon soil with well waters No. SW 11 and SW 15 (single treatment) was significantly different from that with the control treatment, but the residual nitrogen with well water No. SW 15 (split treatment) was not significantly different from that with the control treatment. The residual nitrogen in the IOP, H-Acid and Safety Fuse soils with well water No. SW 15 (single treatment) was not significantly different from that with well water No. SW 15 (split treatment), while the residual nitrogen in the Nylon soil with well water No. SW 15 (single treatment) was significantly different from that with well water No. SW 15 (split treatment).

The nitrogen (%) remaining in soil was calculated from the residual nitrogen in soil. The nitrogen (%) remaining in the soil with well water No. TW 8 was not calculated because the grass died. There was very little nitrogen remaining in all four soils as inorganic nitrogen only 0.2 - 2.46 % of nitrogen applied.

Table 6.14. : The residual nitrogen and nitrogen (%) remaining in the soil after treatment.

Soil and treatment	NH_4-N (mg/kg) in soil	NO_3-N (mg /kg) in soil	NO_2-N (mg/kg) in soil	Residual N (mg /pot) in soil*	N (%) **remaining** in soil
IOP soil (Soil No. 1) :					
Standard N, P, K control (single)	0.64	0.13	0.21	0.59 a	0.38
Well water No. TW 8 (split)	3.20	2.44	0.17	3.49	-----
Well water No. SW 11 (split)	1.58	1.41	0.26	1.95 a	1.24
Well water No. SW 15 (single)	1.56	1.13	0.31	1.80 a	1.15
Well water No. SW 15 (split)	1.04	0.91	0.26	1.33 a	0.85
H-Acid soil (Soil No. 9) :					
Standard N, P, K control (single)	1.57	0.62	0.28	0.86 a	0.55
Well water No. TW 8 (split)	17.34	8.68	0.20	9.18	-----
Well water No. SW 11 (split)	3.11	7.59	0.32	3.86 a	2.46
Well water No. SW 15 (single)	2.07	4.31	0.51	2.39 a	1.52
Well water No. SW 15 (split)	1.82	1.70	0.33	1.35 a	0.86
Safety Fuse soil (Soil No. 11) :					
Standard N, P, K control (single)	0.98	0.00	0.17	0.59 a	0.37
Well water No. TW 8 (split)	7.55	0.92	0.00	5.08	-----
Well water No. SW 11 (split)	0.77	0.04	0.21	0.57 a	0.36
Well water No. SW 15 (single)	0.55	0.00	0.21	0.36 a	0.23
Well water No. SW 15 (split)	0.51	0.00	0.17	0.31 a	0.20
Nylon soil (Soil No. 15) :					
Standard N, P, K control (single)	0.80	0.00	0.21	0.50 b	0.32
Well water No. TW 8 (split)	5.45	2.24	0.04	4.63	-----
Well water No. SW 11 (split)	1.29	1.64	0.09	1.81 a	1.15
Well water No. SW 15 (single)	1.22	0.46	0.21	1.14 ab	0.72
Well water No. SW 15 (split)	0.71	0.50	0.08	0.75 b	0.48

* For each soil, mean values followed by same letter within a column are not significantly different at 5 % level using Tukey LSD test. Treatment with well water No. TW 8 was not included in the statistical analysis.

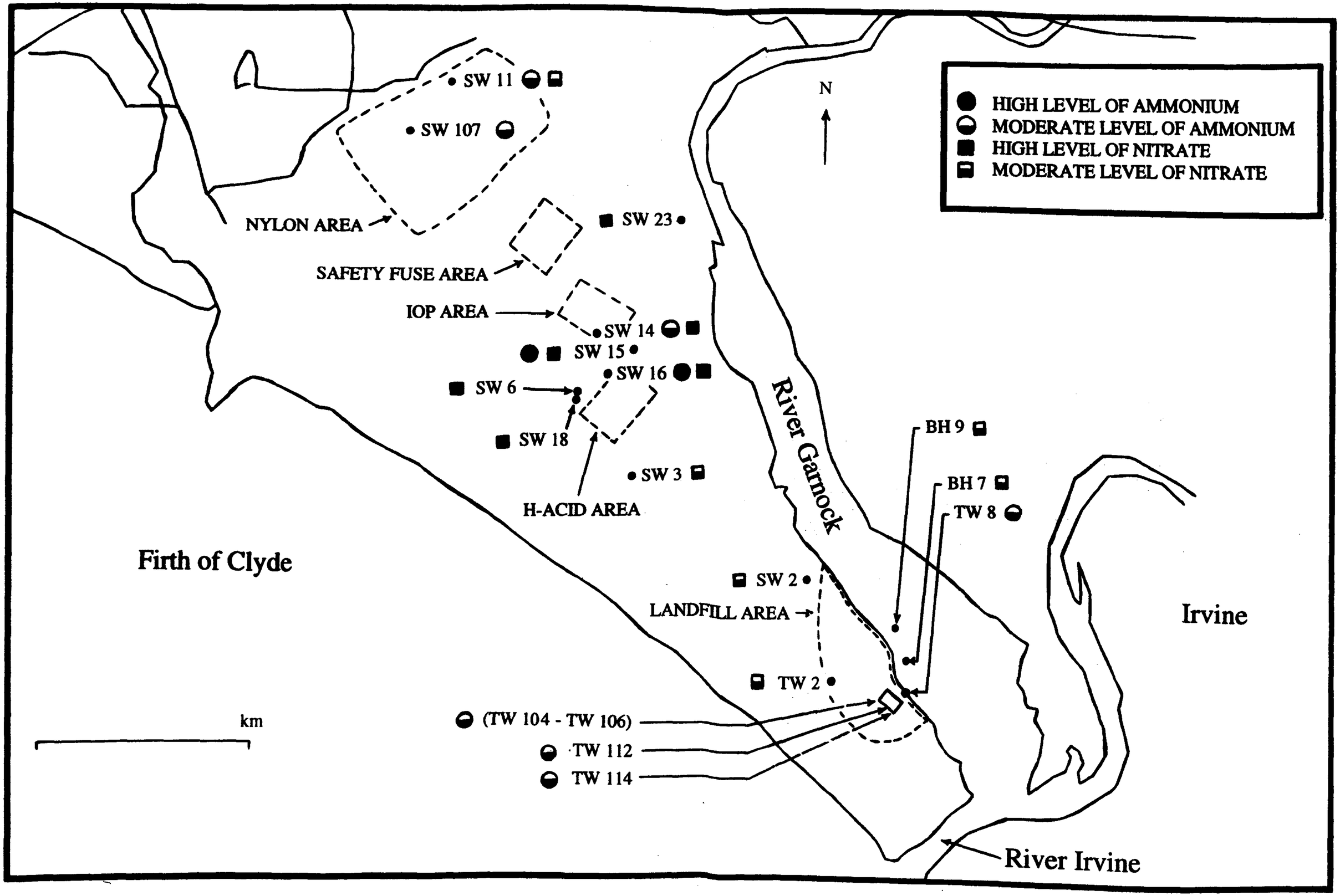
6.4. CONCLUSION FOR THE BIOREMEDIATION OF THE AMMONIUM AND NITRATE CONTAMINATED WATER USING NATURAL SOIL AND VEGETATION

The groundwater quality surveys at the Ardeer site revealed that the majority of the wells had pH in the range from 6.1 to 8.3 but some wells were acidic (pH below 4.3). Ammonium contamination was quite localised at the site. The nitrate contamination was also very closely localised but ammonium and nitrate were not necessarily found together (see Map 6.1.). Chloride, sodium, potassium, calcium, magnesium and iron contamination was widespread at the site. High electrical conductivity was widespread at the site. Well No. SW 15 was the most badly contaminated well on the site.

The soil survey at the Ardeer site demonstrated that the level of nutrients (N, P, K) was low in all four investigated areas (IOP, H-Acid, Safety Fuse and Nylon areas). The levels of toxic metals were low in the soils. Overall, the variations in vegetation cover appear to be due to differences in macronutrient availability rather than any toxicity.

From the preliminary experiment of nitrification, it was concluded that the Safety Fuse and Nylon soils had extremely low nitrification rates and were not suitable for further studies. The Intermediate Oxidation Plant (IOP) and H-Acid soils were suitable for subsequent work together with two garden natural soils (Darvel and Dreghorn) which were used for comparison.

The nitrification experiment showed that well water No. SW 11 showed no inhibition in all four soils. Well water No. SW 15 showed significant inhibition in the IOP, Dreghorn and H-Acid soils but not in the Darvel soil. Well water No. TW 8 significantly inhibited all the soils to the extent that nitrification was barely measurable in the Dreghorn, IOP and H-Acid soils.



Map 6.1. : Groundwater wells with elevated levels of ammonium and nitrate at the Ardeer site.

The results of the pot experiment showed that well water No. TW 8 had a clear negative effect on the growth of grass as the yield of this treatment was the lowest in all four soils in the first period and there was no growth in the second period. Toxicity symptoms appeared on the grass of the pots treated with this well water.

The grass treated with well water No. SW 15 (split treatment) and the control treatment (single treatment) was more efficient in nitrogen uptake than that treated with well water No. SW 15 (single treatment) in all four soils. There was very little nitrogen remaining in all four soils as inorganic nitrogen only 0.2 - 2.46 % of nitrogen applied.

These results of the pot experiment suggest the possibility of applying the bioremediation treatment of the ammonium and nitrate contaminated water in the field at the Ardeer site. A field study should be undertaken to evaluate the efficiency of this bioremediation treatment. This field study would require a suitable uniform area to layout the plots, preferably close to the source of water to be used. In addition, it is necessary to carry out a hydrological survey to determine the following aspects :

1- The size of groundwater reservoir.

2- The rate of removal of the water.

3- The time scale of the water application.

The IOP area is unsuitable because of the varying chemical properties in the topsoils and subsoils. The imported topsoil had varying depth (ranged from 6.0 to 15 cm) and overlying building rubble, concrete , brick, ash, sand and stony rubble. In addition, the bottoming for the IOP building was probably slag from the adjacent former iron works. There are slightly higher levels of barium in the subsoil on parts of the area than is typical of the site. Relative to the ICRCL Guidance Note 59 /83 (1987), the subsoil of the sample site No. 2 exceeded the threshold value for copper and nickel. The subsoil of the sample site No. 1 was on the borderline of the threshold value for zinc (see Map 4.4.).

The imported topsoil on the H-Acid area is overlying concrete, building rubble and sand. The depth varied from 11.0 to 22.0 cm. Relative to the ICRCL

Guidance Note 59 /83 (1987), the topsoil and subsoil of the sample site No. 9 exceeded the threshold value for nickel. Nevertheless, the existing grass cover was uniform and good, therefore, this area may be suitable to be used (see Map 4.5.).

The imported topsoil on the Safety Fuse area is more uniform in depth and generally overlying a sandy subsoil, however, the pH is rather variable and on part of the area the topsoil overlies asphalt rubble in the case of site No. 12 (see Map 4.6.).

The Nylon area is sandy with some development of a natural topsoil. There is concrete and building rubble over a wide area but the rest of the area could be cultivated. Relative to the ICRCL Guidance Note 59 /83 (1987), the topsoil of the sample site No. 17 exceeded the threshold value for copper and was on the borderline of the threshold value for zinc. Therefore, site No. 17 should be excluded from the field study due to the potential toxicity of copper and zinc to grass (see Map 4.7.).

All of the site has some problems of uniformity but carefully selected areas of H-Acid, Safety Fuse and Nylon could be identified to layout a field trial. A background of phosphorus and potassium (magnesium) fertiliser would be required and some areas might require liming. A careful preliminary uniformity test would be necessary.

The groundwater sources with elevated levels of ammonium-N available to be used in this field study are :

Well water No. SW 15 has a very high level of ammonium (8090 mg /l) and well water No. SW 16 has high level of ammonium (117 mg /l). Well waters No. SW 11, SW 14, SW 107, TW 8, TW 104 - TW 106, TW 112 and TW 114 have moderate levels of ammonium (in the range of 17.0 to 58.0 mg /l) [see Map 6.1.].

The groundwater sources with elevated levels of nitrate-N available to be used in this field study are :

Well waters No. SW 14, SW 15 and SW 18 have very high levels of nitrate (in the range of 2701 to 10860 mg /l). Well waters No. SW 6, SW 16 and SW 23 have high levels of nitrate (in the range of 121 to 840 mg /l). Well waters No. SW 2, SW 3, SW 11, TW 2, BH 7 and BH 9 have moderate levels of nitrate (in the range of 14.0 to 45.5 mg /l) [see Map 6.1.].

A suitable groundwater source for a field study in the Safety Fuse and H-Acid areas are well waters No. SW 14, SW 15 and SW 16. These well waters can be added at a low volume as a split treatment which was more efficient in nitrogen uptake than the single treatment.

A suitable groundwater source for a field study in the Nylon area is well water No. SW 11. This well water would have to be added at a high volume as a split treatment.

The irrigation of ryegrass with highly contaminated groundwater could result in problems such as salinity, high or low pH and toxicity. Salinity has an effect on the physiology of plant as it decreases the growth as a result of insufficient water. Well waters No. SW 14, SW 15 and SW 16 have very high electrical conductivity but since they will be added as diluted water they should not cause a salinity problem. Well waters No. SW 11 and SW 107 had low electrical conductivity and so should not cause a salinity problem.

Well waters No. SW 15 and SW 11 have low levels of boron (1.12 and 0.82 mg /l, respectively) and can be added safely without causing potential boron toxicity to grass. The cleaning of well water No. TW 8 from its high level of boron by a chemical method makes the bioremediation treatment too costly. In addition, the iron level in well water No. TW 8 is high (95 mg /l), therefore, well water No. TW 8 should be avoided for this field study.

The climatic conditions such as rainfall, potential evapotranspiration and temperature should be taken into consideration when carrying out the bioremediation treatment in the field as these climatic conditions affect the water requirements and the growth of grass. The water requirements for grass varies from the winter months to the summer months due to these climatic conditions.

The average monthly rainfall, potential transpiration and water requirements for grass at the Ardeer site are presented in Table 6.15. As can be seen, on average, there are two months (May and June) when irrigation could be applied without causing leaching. Overall, 3 cm of irrigation water can be applied in these two months.

Table 6.15. : The average monthly rainfall, potential transpiration and water requirements for grass at the Ardeer site.

Month	Rainfall (mm)*	Potential Transpiration (mm)**	Water requirements (mm)
January	91.01	6.35	-----
February	55.49	13.97	-----
March	58.82	33.02	-----
April	52.16	57.15	4.99
May	56.60	88.90	32.30
June	64.37	95.25	30.88
July	88.79	86.36	-----
August	101.00	69.85	-----
September	110.99	46.99	-----
October	107.66	27.94	-----
November	109.88	10.16	-----
December	103.22	7.62	-----

* Source :
- A NON (1989).
- A NON (1977).

** Source :
- A NON (1967).

There is only one option to apply well waters with moderate levels of nitrogen as follows :

Well water No. SW 11 is used as an example for well waters with moderate levels of nitrogen. Well water No. SW 11 had 83.6 mg /l total nitrogen would have to be applied at a high volume (300 m^3) to provide 25.08 kg N /ha. This required volume (300 m^3) from well water No. SW 11 was calculated from the following equation :

Required volume for 1 hectare = 100 m × 100 m × 0.03 m = 300 m^3

Where :

1 hectare = 100 m × 100 m.

3 cm = 0.03 m (see Table 6.15.).

The total nitrogen to be provided from well water No. SW 11 was calculated from the following equation:

$$\text{Total nitrogen (kg /ha)} = \frac{\text{Required volume for 1 hectare} \times \text{Total nitrogen (mg /l)}}{1000}$$

Where :

mg /l = g /m^3

There are several options to apply well waters with very high levels of nitrogen as follows :

Well water No. SW 15 is used as an example for well waters with very high levels of nitrogen.

First option :

The first option of applying well water No. SW 15 is to apply it at a low volume. Well water No. SW 15 had 16190 mg /l total nitrogen can be applied at a low volume (6.18 m^3) to provide 100 kg N /ha. This required volume (6.18 m^3) from well water No. SW 15 was calculated from the above equation used for calculating the total nitrogen (kg /ha).

The advantage of applying well water No. SW 15 at low volume (6.18 m^3) is that this application can be applied at wet time in the year, however, the disadvantage is that this application is too small to apply.

Second option :

The second option for applying well water No. SW 15 is to blend well water from more than one well using well water No. SW 15, e.g., blending well water No. SW 11 with well water No. SW 15 to achieve a realistic irrigation rate at a suitable nitrogen level.

Third option :

The third option of applying well water No. SW 15 might be to overirrigate in expectation that the ammonium would be retained in the soil. This is more likely to work with the H-Acid area (59.0 c $mole_c$ /kg CEC) but not with the other three areas; IOP, Safety Fuse and Nylon areas (CEC ranged from 7.1 to 14.3 c $mole_c$ /kg). The subsoil of the H-Acid area is sandy and permeable, therefore, waterlogging is not expected.

GENERAL CONCLUSIONS

The current study was accomplished to investigate groundwater contamination and bioremediation treatment for cleaning up groundwater contaminated with ammonium and nitrate using natural soil and vegetation [perennial ryegrass (*Lolium perenne* L.)] at an contaminated industrial site, the Ardeer site. The study was carried out using well waters from the suspected areas and soil samples from the selected areas at the site.

The developed methods for measuring ammonium in soil and water, measuring low levels of ammonium in groundwater (< 0.1 mg /l) and decolorising the highly coloured groundwater samples were used to help the experimental work of the study. The investigation of groundwater quality at the Ardeer site helped to identify the sources of nitrogen contamination, to locate the distribution of ammonium-N, nitrate-N and other contaminants, and to evaluate the groundwater quality parameters. The soil survey at the Ardeer site helped to choose soil samples suitable for nitrification incubation experiment and pot experiment. The nitrification experiment helped to assess the effects of groundwater composition on the nitrification rate in soil and to determine the ability of soils to nitrify ammonium and thus promote leaching as nitrate. The pot experiment was undertaken to evaluate the efficiency of the perennial ryegrass (*Lolium perenne* L.) in the remediation of high ammonium and nitrate groundwater. The results of the pot experiment were successful.

The overall conclusion of this study is, therefore, the bioremediation treatment of the ammonium and nitrate contaminated water can be applied in the field over a period of years. A field study should be undertaken to evaluate the efficiency of this bioremediation treatment. This field study would require a suitable uniform area to lay out the plots, preferably close to the source of water to be used. In addition, it is necessary to carry out a hydrological survey to determine the following aspects :

1- The size of groundwater reservoir.

2- The rate of removal of the water.

3- The time scale of the water application.

The climatic conditions such as rainfall, potential evapotranspiration and temperature should be taken into consideration when carrying out the bioremediation treatment in the field as these climatic conditions affect the water requirements and the growth of grass. There are three options to apply the contaminated groundwater as follows :

1- To apply the contaminated groundwater at low or high volume depending on its level of nitrogen.
2- To blend well water with high level of nitrogen with well water with low level of nitrogen to achieve a realistic irrigation rate at a suitable nitrogen level.
3- To overirrigate in expectation that ammonium would be retained in the soil.

The ryegrass used in this bioremediation treatment can be disposed of by the incineration and landfilling the ash or landfilling the grass.

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