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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

*In the name of God, the Mercy-giving, the Merciful!*

مَنْ لَمْ يَشْكُرِ الْمَخْلُوقَ لَمْ يَشْكُرِ الْخَالِقَ

*“One who cannot appreciate the created, cannot appreciate  
the Creator”*

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## Summary

The work presented in this thesis contains a case study of a former industrial site, which has been contaminated with chromium. During this study some methods were required to be developed and evaluated for the analysis of  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N in soil and P in plant.

There were some problems during colorimetric analysis of nitrite-N and nitrate-N in chromium contaminated soils extracts. The systems were sensitive to the first addition of chromium. Effort has been made to solve these problems using alternative reagents containing 1mg/l Cr(VI) instead of conventional reagents in order to maintain a constant low level of chromium in the systems while analysis of the extract solutions was in progress.

During phosphorus determination in certified plant digests there were also problems which were both physical and chemical in the molybdate/metavanadate colorimetric analysis system. Experiments were carried out to identify the problems and their solutions. It was shown that the yellow colour of digest solutions stuck to the PVC tubing but did not stick to the glass or the silicon rubber tubing. The molybdate-metavanadate method of analysis happened to give lower values compared with the ascorbic acid method in low pressure digest solutions due to chemical interference which happened during the analysis.

A comparative study was made to evaluate seven digestion and two analysis methods for phosphate-P determination in vegetation samples. It was shown that the perchloric acid digestion method is a suitable method for phosphorus and heavy metals analysis in plant material. Moreover since the ascorbic acid method was carried out without problems in this experiment it was considered suitable for use for phosphorus analysis in the digest solutions.

Study of a former industrial land, which was contaminated with chromium, was the major research topic of this study.

A survey study of the land was made to evaluate the pH, electrical conductivity and percentage of organic matter of soils, available nutrients, total nutrients, available

and total chromium and total other heavy metals in the soils. The survey also includes the total nutrients, total chromium and some other heavy metals in the plants and root mat.

Soil and plant samples were collected at different times. The levels of total nutrients in soils and plants, the plant available nutrients in soil, total heavy metals in plants and soil extractable chromium were measured in the soil and plant samples at each time. The grass yields were calculated for each sampling point at each time and the values were plotted against time to demonstrate the changes in yield, nutrient and extractable chromium of the site during the different time intervals.

Soils throughout the site had high total chromium concentrations, high pHs and low plant available nitrogen and low vegetation yields. However, chromium was not detected in the plants.

The effects of fertilizer additions were evaluated in two pot experiment studies of these soils using ryegrass as a test crop. The first pot experiment was carried out to look at the response of the soil samples to different combinations of N, P and K fertilizers to determine which fertilizer is limiting the plant growth. The response was measured by dry matter production. Nitrogen was shown to be the limiting factor for growth of the grass in these soils.

Alleviating the limiting factor resulted in a yield increase. Since the trend of this increase can not be fully applied to all types of soils and spoils due to differences in nature of the material a second pot experiment was carried out to look at the response of the vegetation to addition of the different rates of nitrogen fertilizer.

The results showed that the application of 100 /50 /50 of N/P/K kg/ha fertilizer could obtain the maximum yield for the site except for a soil sample from the base of the east side (sample 4), which did not respond to more than 50 of N kg/ha. Since the changes in the yields from these experiments were not affected by soil extractable chromium in the two experiments the possibility of the direct effect of chromium on plant growth was discounted.

The occurrence of the nitrogen turnover and possible toxicity effects of chromium on these processes was assessed during a number of incubation studies. First of all the nitrogen supply power of the soils was evaluated using an incubation test for the nitrogen mineralization rate of the soils from this site. This experiment was done



under aerobic conditions. An attempt was also made to look at possible volatilization of ammonia due to the high pH of these soils.

Total inorganic-N decreased during the first week of the incubation. Some soils, which are on the east side of the site (one on the slope and another on the base of the slope) showed no mineralization during the 17 weeks incubation period. Two other soils showed slow mineralization after a 10 days lag period and two of them showed reasonable mineralization rates compared with other grassland soils. The results showed any ammonium released is immediately converted to nitrate-N and there is no loss of ammonia gas due to non detectable ammonium-N in these 17 weeks mineralization experiments.

In the incubation study of the nitrification of applied ammonium-N to the soil samples from different sampling points of the site, nitrogen loss was seen to happen in the first few days of incubation. Both ammonia gas volatilization and inorganic-N immobilization happen to be the cause of the nitrogen loss at the first part of the incubation. Nitrification has been shown to take place in these soils after a lag period. The length of the lag period was different in different soils and negatively correlated with available-P ( $r=-0.952$ ).

# CHAPTER ONE

## INTRODUCTION

There is increasing concern about our natural environment and the need to protect it for the benefit of future generations. The natural environment consists of air, water and land. Pollution of the environment is due to release of any substance or energy liable to cause hazards to human health, harm to living resources or ecological systems. Contaminated land is a land which represents an actual or potential hazard to health or the environment as a result of current or previous use (Attewell, 1993).

Alloway (1995) pointed out that in practice, the terms “contamination” and “pollution” are frequently used interchangeably, although pollution is usually more pejorative. He added that for soils there is a fairly wide general agreement to choose and follow the use of the term “contamination” for any situation in which elevated concentrations of a substance occur. A contaminant of the environment in general, and soil in particular, are heavy metals. Heavy metals, or trace metals, are a large group of trace elements which are both industrially and biologically important (Alloway, 1995).

Excessive concentrations of metal in soil can be associated with naturally occurring ore deposits, but more usually are the results of human activities. Human activities can affect the natural processes in soil, water and the atmosphere and cause problems. Some of the environmental problems associated with the soils are chemical or physical (Alloway, 1995).

Trace elements, including all the micronutrients at high concentrations in soil, are able to damage plant and microorganism growth. The elements which potentially are toxic to plant and human are: As, Be, Sb, Cd, Cr, Pb, Hg, Ni, Se, Ag, Ti, Cu and Zn (McBride, 1994). In some cases the availability of these ions for plant uptake or leaching into groundwater is an important environmental issue because heavy metals in

elevated concentrations are toxic for human and other living organisms and uptake and leaching is a pathway for these metals to be moved to human environment.

A former industrial site, which is contaminated with chromium, is the study area for this project. The poor vegetation and poor establishment of trees in the central part of the site on the spoil heap of the area led to the investigation of this site. The probable toxicity of chromium to the plant growth and other factors such as major nutrient availabilities and limitation of the soil i.e. nitrogen and phosphorus are underlying aims to be investigated in order to provide a good vegetation cover on the soil.

## 1.1 Chromium (Cr)

Chromium is the 21st, most abundant element in the earth's crust with concentration of 100µg/g. It is the seventh highest in the whole Earth with 3700µg/g concentration (Kats and Salem 1994, Alloway 1995). Chromium is found in ultramafic and mafic rocks (Nriagu, 1988).

Chromium was first isolated from crocoite, an attractive rare mineral from a Siberian gold mine, not from chromite from which chromium is recovered economically (Nriagu, 1988). The mineral, *plomb rouge de siberie* from the Beresof gold mine in Siberia, was analyzed and described by Johann Gotlob Lehmann a former Prussian mining official and professor of chemistry of St. Petersburg in 1766. The mineral is naturally a lead chromate and is now known as *crocoite* with a bright red-orange colour (Nriagu, 1988). In 1794 Nicolas-Loius Vauquelin and L.C.H. Macquart, French chemists analyzed the mineral and said that the mineral contained lead, iron, aluminium, and large amount (nearly 38%) of oxygen. After three years Vauquelin described that the lead was joined to an unusual acid and that he could isolate the metal by heating the mixture of acid and carbon in a graphite crucible. He named this new metal *chrom* from the Greek word *χρῶμα* because of its brilliant colour. He noted that the new metal would find few uses because of its infusibility (Nriagu, 1988).

The chromium accumulation in the serpentine rocks of Saxony was found in 1800. Soils associated with serpentine rocks are often comparatively barren of vegetation and high in chromium content, but it is considered to be nickel toxicity rather than

chromium toxicity. The average chromium content in world soils is 84 mg/kg while a wide range of chromium (0.3-10,000) is found in different soils (Alloway, 1995).

Chromite  $\text{FeOCr}_2\text{O}_3$  is now the major economically important source of chromium. It is a dense, black mineral consisting primarily of iron(II) and chromium(III) oxides, with magnesium and aluminum oxides present in lesser amount. It has different grades, due to the  $\text{Cr}_2\text{O}_3$  content of the ore. Different grades are used for different industrial purposes (Kats and Salem 1994).

### **1.1.1 Uses of Chromium**

#### **1.1.1.1 Chemical**

Chromium salts have been used as pigments since the discovery of crocoite. Different colours were produced such as: violet, red, yellow, green, orange and even blacks gray and brown to use in painting, enameling and colouring arts. Chromium was also used as a mordant to fix organic dyes on fabric especially wool. It is also used as a tanning agent precipitating as oxides on leather surfaces (Nriagu, 1988; Kats and Salem 1994).

#### **1.1.1.2 Refractories**

Chromate is also used for refractory materials. Various types of bricks were introduced with different ratio of chromate-magnesite with enhanced physical and chemical features (Nriagu 1988; Kats and Salem 1994).

#### **1.1.1.3 Alloys**

Chromium was used for electroplating of objects from long ago, with continuous method development. It is used for making alloys such as high-carbon ferrochromium and low-carbon ferrochromium. In chromium containing steel, chromium provides increased hardness, improved corrosion resistance and altered magnetic properties.



Because of its resistance to the action of oxygen, in metallurgy, it was used with nickel to increase resistance of iron against attack of hot gases. It was also used in different alloys such as iron-silicon-chromium and non ferrous alloys like chromium–cobalt–nickel, chromium–cobalt–molybdenum or tungsten. These non ferrous alloys are now widely used in rock–cutting tools (Nriagu, 1988; Kats and Salem, 1994).

### 1.1.2 Chemistry of Chromium

In order to study the toxicity of chromium it is necessary to understand the chemistry, biochemistry and biologic function of chromium compounds. Chromium exists in different oxidation forms with different stability. The environmental chemistry of chromium compounds involves oxidation-reduction transformations, precipitation-solubilization reactions, and adsorption-desorption phenomena (Kats and Salem, 1994).

In compounds, chromium demonstrates oxidation numbers (II), (III), (IV), (V) and (VI). Compounds of Cr(II) are vulnerable to air oxidation and it is a strongly reducing form and easily gives up an electron and oxidizes itself to Cr(III). Cr(IV) reacts as a metal ion and Cr(V) is  $\text{CrO}_4^{3-}$  with few stable compounds. Cr (IV) and Cr(V) are transient intermediates in the reduction of hexavalent chromium (i.e.,chromate) to the trivalent state. Cr(III) is the most stable form of chromium in solution. Cr(VI) i.e  $\text{CrO}_4^{2-}$  and  $\text{Cr}_2\text{O}_7^{2-}$  are strongly oxidizing and unstable in acid solution in the presence of electron donors (Nieboer and Jusys, 1988). Elemental Cr is never found in nature (Pacyna and Nriagu, 1988). Cr(III) and Cr(VI) are the two forms of chromium which are found in the nature.

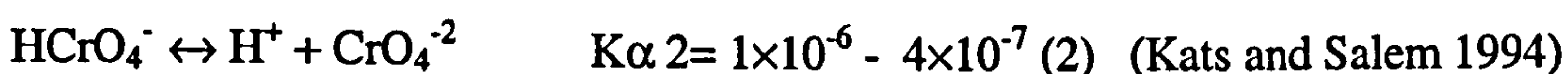
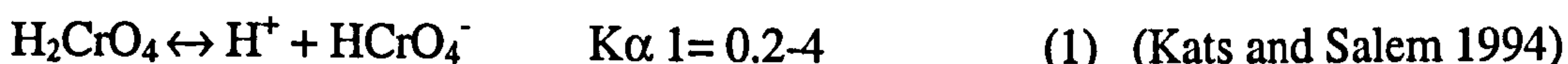
#### 1.1.2.1 Cr(VI)

Cr(VI) is strongly oxidizing acid and exists as  $\text{CrO}_3$ (anhydrite chromium trioxide),  $\text{CrO}_4^{2-}$  (chromate) and  $\text{Cr}_2\text{O}_7^{2-}$  (dichromate).  $\text{CrO}_3$  is highly soluble in water and gives yellow chromate ion. In the acidic medium it gives different pH and concentration dependant species (Nieboer and Jusys, 1988).

In the basic solution above pH 6  $\text{CrO}_3$  forms the tetrahedral yellow *chromate* ion  $\text{CrO}_4^{2-}$ ; between pH 2 and 6,  $\text{HCrO}_4^-$  and the orange-red dichromate ion  $\text{Cr}_2\text{O}_7^{2-}$  are in equilibrium; and at pH values  $< 1$  the main species is  $\text{H}_2\text{CrO}_4$  (Cotton and Wilkinson, 1988).

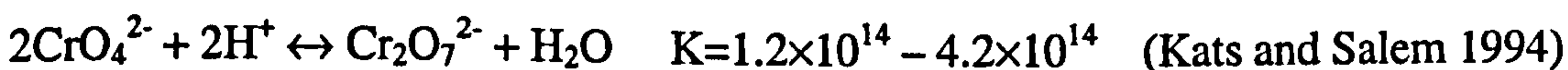
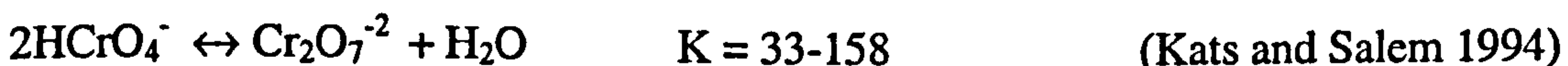
Its dissociation is as follow:

$\text{H}_2\text{CrO}_4$  is a strongly acid with pK values measured of (pK= 0.2-4).



With regard to equation (2) it can be noticed that the monohydrogen chromate ion ( $\text{HCrO}_4^-$ ) is a weak acid.

At higher concentrations ( $> 10^{-2}\text{M}$ ) and low pH,  $\text{HCrO}_4^-$  gives the dichromate ion.



It is generally agreed that in the natural systems at concentrations lower than  $10^{-2}\text{M}$ , dichromate is dominant below pH 3, and  $\text{HCrO}_4^-$ , is dominant above pH 3.

The solubility of chromate salt is complex and pH dependent and it may be divided to three groups:

a- Sparingly soluble to insoluble such as the chromates of zinc, lead ( $1.8 \times 10^{-14}$  mol/kg water), barium ( $2 \times 10^{-10}$  mol/kg water), silver and strontium.

b- Intermediate solubility for example calcium chromate ( $7.1 \times 10^{-4}$ ), hydrated calcium chromate (0.83 mol/kg water), and potassium dichromate (0.44 mol/kg water).

c- High solubility sodium chromate (5.2 mol/kg water) and potassium chromate (3.3 mol/kg water) as well as sodium dichromate (9.7 mol/kg water) and calcium dichromate.

It can be said species with high solubility are powerful oxidizing agents (Nieboer and Jusys 1988; Kats and Salem, 1994).

### 1.1.2.2 Cr(III)

The chromium(III) ion is the most stable form with a low affinity for the oxide and hydroxide ions. The hydrated Cr(III) ions,  $\text{Cr}(\text{OH}_2)_6^{+3}$ , can hydrolyze to hydroxide ion and then release of proton. Different species can be found at different pH. At pH: 4, about one-half of the hydrated Cr(III) ions are as monohydroxo complex,  $\text{Cr}(\text{OH})^{+2}$  while  $[\text{Cr}(\text{H}_2\text{O})_6]^{+3}$  dominates in acidic conditions under pH 4.

Cr(III) has the ability to combine with some ions containing ligands. The stability of Cr(III) complexes is in the order:  $\text{F}^- > \text{Cl}^- > \text{Br}^-$  and O ligand  $>$  S ligand (Nieboer and Jusys 1988).

Chromium(III) oxide ( $\text{Cr}_2\text{O}_3$ ) both unhydrated and hydrated are insoluble. Chromium(III) phosphate is also insoluble. The solubility of anhydrous forms of complex with common anion like  $\text{CrBr}_3$ ,  $\text{CrCl}_3$ ,  $\text{Cr}_2(\text{SO}_4)_3$  are low, while the hydrated forms of them are also soluble. Salts of acetate, oxalate and nitrate are also soluble (Nieboer and Jusys, 1988).

Figure 1.1 illustrates the Eh – pH diagram for chromium. It provides a generalized representation of dominant aqueous species and their redox stabilities. This is for conditions of chemical equilibrium. In the natural environment or soil system chromium may undergo changes in oxidation state if the redox conditions of the soil or environment are altered (Rai et al., 1989).

Oxidised

Reduced

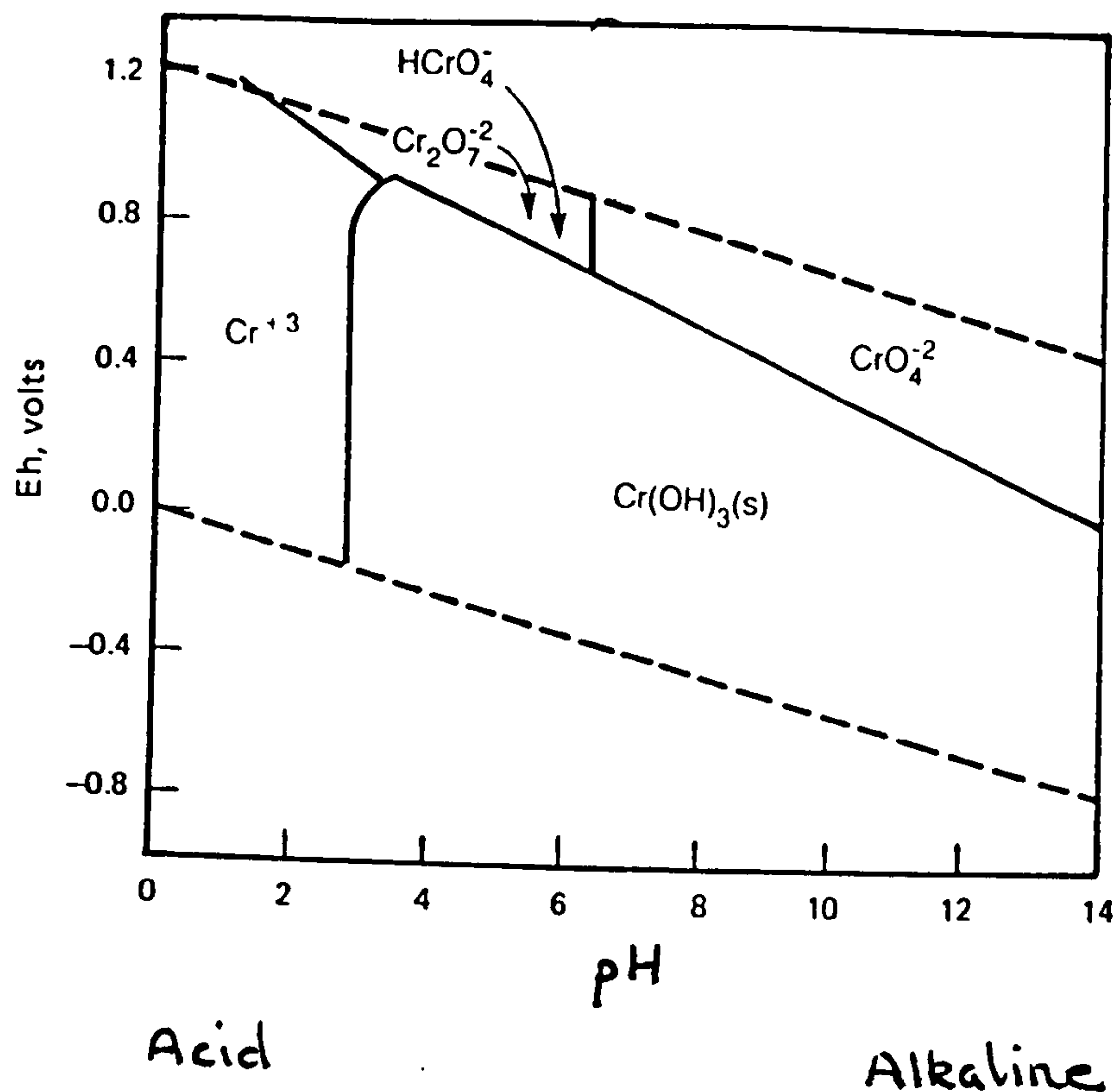


Figure 1.1: The Eh – pH diagram for the various chromium species. (Rai 1989; Kate and Salem, 1994)

### 1.1.3 Chromium in Soil

#### 1.1.3.1 Solubility and Mobility

Most of the Cr(III) in soils has low solubility and reactivity. Cr(III) can form complexes with some organic ligand such as fulvic acid, citric acid, diethylene triaminepentaacetate (DTPA). The Cr(III) complexes are soluble above pH: 5.5, while non complex Cr(III) is precipitated above this pH (James and Bartlett, 1983a). They also mention that liming the soil decreases the extractable Cr(III), as liming forms inorganic hydroxides from Cr(III) and prevents organic complex formation. It can be



said that ionic Cr(III) at below pH 5 is chemically and biologically the most available forms of inorganic Cr(III). The soluble organic, some florid complexes and some inorganic material are present in soils in a size of colloid (Bartlett and James, 1988).

Hexavalent chromium compounds are more soluble than trivalent chromium compound under most environmental conditions. Dissolved chromium in the geologic and aquatic environments is usually hexavalent chromium. Cr(VI) can be leached through the soil profile. Shallow groundwater especially in sand and gravel aquifers may have extensive concentration of Cr(VI) while in clay aquifers low amounts of Cr(VI) is expected due to their difference in groundwater velocities (Calder, 1988; Kats and Salem, 1994).

### **1.1.3.2 Oxidation and Reduction**

It was thought that the oxidation of Cr(III) does not occur in soil. (Bartlett and Kimble, 1976a). They reported no evidence of chromium oxidation in their study regardless of pH, aeration, moisture, level of Cr(III), etc. Bartlett and James, (1979 and 1988) stated that a portion of Cr(III) as a salt or hydroxide which is added to any soil, is oxidized to Cr(VI), at pH above 5 and if the soil sample is fresh and field moist. They noted that the presence of oxidized manganese in the soils serves as the electron link between the added Cr(III) and oxygen consequently encouraging Cr(III) oxidation.

Temperature and pH are also factors affecting oxidation of Cr(III). At low temperature the reaction is slowed while at higher temperature oxidation will increase. At high pH trivalent chromium is readily oxidized to the hexavalent state.

Low molecular weight organic acids can increase solubility and mobility of Cr(III), makes it ready for possible oxidation. This may occur in sewage sludge or tannery waste amended soils. However Cr(III) complexes with high molecular weight organic matter ligands are not easily dissociated and oxidized (Bartlett and James, 1988; Kats and Salem, 1994) .

Cr(VI) can be reduced in the soil system. The factors, which affect the reduction process, are organic matter and pH. Soils with high organic matter have more capability of reduction of Cr(VI) (Bartlett and James 1988; Kats and Salem, 1994). Organic matter serves as organic form of energy and electron donor while there are also other electron donors such as  $\text{Fe}^{2+}$  and  $\text{HSO}_3^-$  (Kats and Salem, 1994). The reduction of Cr(VI) in a soil containing organic matter can be more at lower pH compared with the same soil with higher pH and there is no reduction in acidified organic-free soil (Bartlett and Kimble, 1976b; Bartlett and James, 1988; Bartlett, 1991) and more with ferrous ion (Rai et al., 1989). In a study carried out by James and Bartlett (1983b), they found more reduction in unlimed soil (pH: 5.3) compare with a limed soil (pH: 6.5).

### **1.1.3.3 Adsorption and Exchange**

Cationic trivalent chromium is usually strongly adsorbed by the soil particles with negative charge such as clay minerals and organic matter in soils.

The pH of the soil can effect adsorption and precipitation as well as reduction. It affects not only the speciation of Cr, but also the surface charge of the soils, in term of quantities of positive and negative charge on soil colloids especially Al(III), Fe(III), Mn(II, IV) oxides and organic matter (Bartlett and James, 1988). James and Bartlett (1983c) found that liming the soils decreases the exchangeable Cr(VI). They mentioned this is probably because liming increases the pH and then decrease the positive charge of pH dependant charge fraction (oxides, kaolinite, and organic matter) in soils.

$\text{CrO}_4^{2-}$  is the dominant species of Cr(VI) at above pH 6 due to dissociation of  $\text{HCrO}_4^-$ . It can be adsorbed as an exchangeable ion on positively charged surfaces of aluminum and iron oxides by ligand or form complexes or held tightly in soils like phosphate or stay soluble in soil solution. The phosphates can compete with chromate for soil site to adsorb and cause its solubility and subsequent reduction.

### **1.1.4 Bioavailability of Chromium**

Cr(VI) is a highly soluble anion and can be readily penetrate into the cell and be absorbed by living organisms. Once anionic chromium Cr(VI) has moved to the plant roots and been absorbed by them, most of it is reduced to Cr(III) and retained by the roots in a tightly bound or insoluble form or in a soluble organic complex that is not translocated to the tops. Therefore the concentration of Cr in the roots is much more than that of the shoots (James and Bartlett, 1984b).

They found that the levels of chromium of root were highly correlated with soluble Cr(VI) concentration in soils amended with Cr(III).

The soluble form of Cr(III) for biological system is organically complexed with low molecular weigh organic matter. It probably does not penetrate membranes (Bartlett and James, 1988). This is while plants in culture solution containing up to 2mg Cr(VI)/l showed dose dependant uptake and accumulation of Cr both in roots and leaves (Kats and Salem, 1994).

Low solubility of natural soil chromium compounds limits the forms available to plants to very small fraction of the total concentrations. For this reason, the chromium concentration in the foliar parts of plants shows little relation to the total chromium concentration of the soils in which they grew. Shoot or leaves usually show low level or non detectable Cr. Higher concentrations of Cr were observed in some studies on plants growing on chromate waste mine spoil in which the more Cr(VI) form predominates (Alloway, 1995).

Concentrations of Cr in the foliar parts of the plants growing on non-contaminated soils are less than 1mg/kg, while plants growing on chromium wastes might have higher concentrations (10-190mg/kg).

## **1.1.5 Chromium Contamination**

### **1.1.5.1 Air**

Chromium has been known as a pollutant of the environment despite its role in human metabolism.

There is an interest understanding Cr emissions into the atmosphere, which are a major pathway for Cr transfer between different ecosystems. The largest total amounts of chromium released to the atmosphere are from human activities such as metallurgical industries and refractory brick production. Windblown soil and volcanic activity are the major natural resources of chromium in the atmosphere. Values measured varied from 0.09-1.2ng/m<sup>3</sup>. Any increase of bioavailable chromium to the atmosphere should be considered as a potential hazard for fauna and flora (Nieboer et al., 1988; Alloway, 1995).

### **1.1.5.2 Water**

The contamination of groundwater by chromium can also be important in industrial areas. Plating industries, wood treatment and tannery facilities and chromium mining can be source of discharge of contaminated material. Shallow groundwater especially in sand and gravel aquifers may have extensive concentration but clay aquifers low concentration due to their difference in groundwater velocities (Calder, 1988). Chromium can also enter surface water and is toxic to fish. Gill injury and is considered one aspect of chromium toxicity (Holdway, 1988).

### **1.1.5.3 Human**

Chromium is an essential element for animal and human nutrition. It has an important role in normal carbohydrate, lipid and nucleic acid metabolism.



Chromium in elevated concentrations is toxic. Its toxicity is of great concern because of its affect on human in different ways. Human exposure to chromium compounds is by ingestion of food and water, by inhalation of airborne particles and by contact with numerous manufactured items containing chromium or its compounds (Kats and Salem, 1994).

It has been said that chromate can cause lung cancer as well as mutations and inflict chromosomal damage (Yassi and Nieboer, 1988). Chromium can also cause skin ulceration; acute, subacute or chronic injuries to the nasal tissues and has affect on respiratory system on exposed workers and cause reduction of fertility and birth problems (Nieboer and Yassi, 1988). Chromium compounds can cause skin allergies. It is the second most common skin allergen after nickel (Hainess and Nieboer, 1988).

## 1.2 Nitrogen (N)

Nitrogen is one of the major elements, which is necessary for plant growth. It is very mobile element circulating between the atmosphere, the soil and living organisms. In soil ecosystems, which are not disturbed by human activity, such as forest or wild lands, the nitrogen cycle is almost balanced. A portion of ammonium from nitrogen mineralization is nitrified to nitrate, and most of the mineral nitrogen transferred to the biomass. Some nitrate can be leached in saturated soils with rainfall and some undergoes denitrification. In a balanced system the small amount of nitrogen loss is compensated by atmosphere input and nitrogen fixation (Paul and Clark, 1996).

The soil nitrogen cycle consists of two small cycles (Figure 1.2):

- 1- the cycle which includes the processes, related to conversion of atmospheric nitrogen (dinitrogen) into organic nitrogen or microbial biomass (N fixation) and return of fixed nitrogen to atmospheric dinitrogen (denitrification).
- 2- the cycle which include those processes responsible for conversion of the organic nitrogen to inorganic or plant-available nitrogen such as mineralization, nitrification and ammonification and return process of inorganic nitrogen to organic, directly to plant and microorganisms and indirect to animals i.e. immobilization (Tate, 1995).

The majority of soil nitrogen is in organic forms. Soil organic nitrogen like organic matter and biomass nitrogenous compounds are not mobile which can be considered as an advantage for soil nitrogen reservoirs. The amount of soil inorganic nitrogen like nitrate and ammonium changes with time in the soil system. Ammonium can be fixed in clay minerals therefore some of the ammonium can be considered as a nonmobile nitrogen in soil. The soil inorganic nitrogen is only a few percent of total soil nitrogen. Organic nitrogen in soil indicates plant biomass production potential in ecosystem (Tate, 1995).

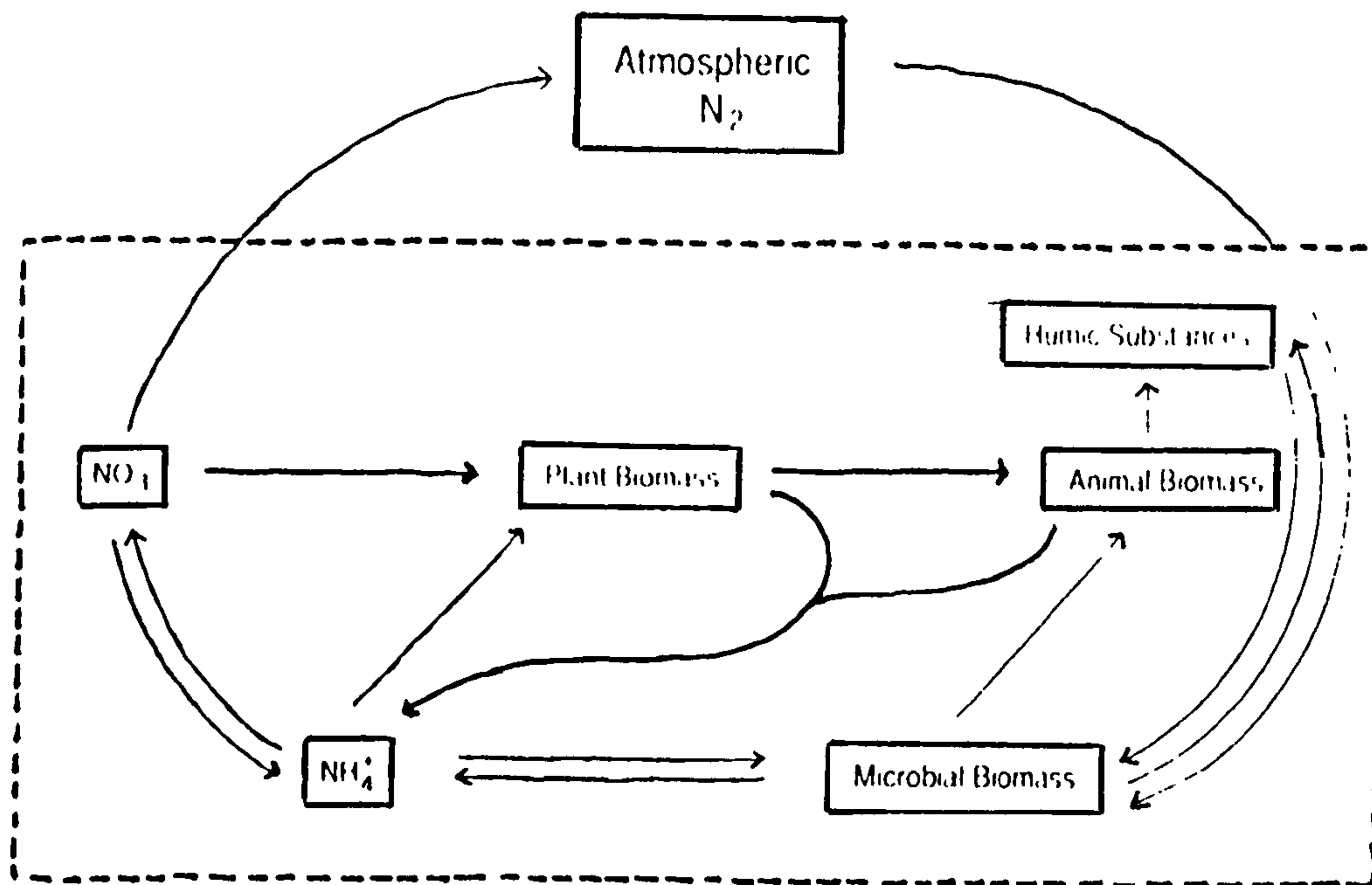


Figure 1.2: Conceptual model of the nitrogen cycle. Nitrogen reservoirs enclosed in the dashed lines in soil, whereas dinitrogen is replenished from atmosphere sources (Tate, 1995)

The availability of soil mineral nitrogen controls plant biomass productivity, provided that other potential factors of synthesis of organic matter such as soil moisture, phosphate, the level of trace mineral and any other soil physical and chemical properties are optimum. The process contributing to the soil nitrogen balance is represented in Figure 1.3.

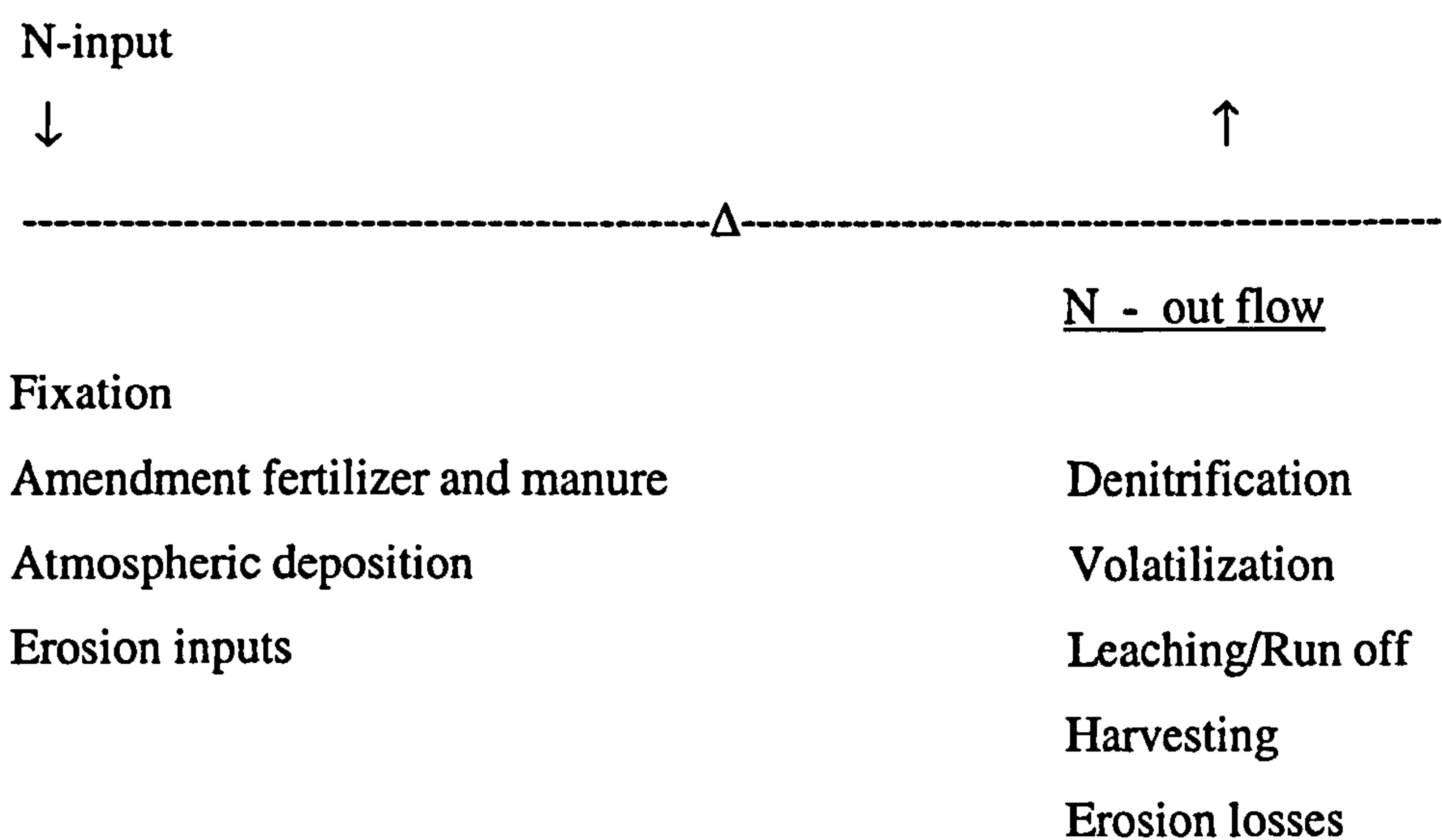


Figure 1.3: processes contributing to soil nitrogen balance (Tate, 1995).

The processes by which the nitrogen is lost from soils are denitrification, volatilization, leaching, harvesting plant by animal, or cropping in agricultural soil systems and soil erosion. In a balanced ecosystem atmospheric deposition, erosional deposition, plant biomass and fixation compensate these nitrogen losses. In case of modifying one of above process, a new biomass productivity will be established. If net nitrogen loss continue the production of biomass will decline. A knowledge of role of nitrogen mineralization is essential in management for production of the plant on a soil ecosystem (Tate, 1995).

### 1.2.1 Nitrogen Mineralization

Nitrogen mineralization is the conversion of organic nitrogen to inorganic nitrogen. In the soil system ammonium-N can be easily oxidized to nitrate so ammonium plus nitrate are estimated as the quantity of soil nitrogen mineralization. Ammonification is a more limited term, which is used for the conversion of organic nitrogen to  $\text{NH}_4^+$ . Soil

organic nitrogen is contained in humic substance, plant material, microorganism and animals.

Nitrogen mineralization rates are determined by the amount of organic nitrogen compounds in soil, which are readily metabolized. Nitrogen mineralization may vary with temperature, moisture, accessibility of the substances to the microorganisms, addition of plant derived organic matter, improvement of soil physical properties and increasing moisture preservation. As it can be concluded, organic nitrogen is mineralized to mineral (inorganic) nitrogen where life is possible. In waterlogged condition in soils the accumulation of organic matter occurs. In this situation plant production is in balance with the slow nitrogen mineralization (Tate, 1995; Paul and Clark, 1996).

Nitrogen mineralization in soil has big role to plant biomass production. It must be mentioned that all mineral nitrogen that produces from this process is not available to the plant. Some of the mineral nitrogen may be denitrified and a part of it may be leached from the soil.

The following equation can demonstrate the changes in soil mineral N.

$$\Delta N_i = N_m - (N_p + N_a + N_d + N_l + N_v + N_f) \quad \text{where:}$$

$N_m$ : Mineralized inorganic nitrogen

$N_p$  : Nitrogen absorbed by plant

$N_a$  : Nitrogen assimilated by microbes

$N_d$  : Nitrogen denitrified

$N_l$  : Nitrogen leached from the soil

$N_v$  : Nitrogen volatilized from the soil

$N_f$  : Nitrogen fixed by clays in soil as  $\text{NH}_4^+$



For the parameters in above equation, it can be understood that different nitrogen pools under the field situation can affect the changes of nitrogen in soil. These changes can be simplified in laboratory incubated soil samples for estimation and assessment of nitrogen mineralization. In the laboratory incubation, experiment is done in the absence of plant growth, and there is no leaching. Although there may be some anaerobic microsites in field incubation where denitrification might occur but this should be minimum in controlled laboratory incubation (Tate, 1995).

In this case net nitrogen mineralization would be:

$$\text{Net } N_m = \Delta N_I = N_m - N_a$$

Nitrogen mineralization is essential for biomass production. In a soil system containing organic matter such as plant and animal biomass, microorganisms and any other organic matter, plant productivity can be controlled by the balance between immobilization and mineralization of nitrogen, that is the net mineral nitrogen which is available for plant uptake.

Microorganisms in soils assimilate mineral nitrogen such as  $\text{NH}_4^+$  and or  $\text{NO}_3^-$  for their activities and life. This process is called immobilization, which can occur under different condition with different soil microorganisms (Tate, 1995).

The activity of the soil microbial community affects the amount of mineral nitrogen assimilation by the soil microbial population. In aerobic soils this process is controlled by compounds, which provide carbon and energy to the active soil microbial community. If these compounds contain sufficient nitrogen to meet the needs of the soil microorganisms, nitrogen deficiency does not occur. The impact of carbon-rich material on available mineral nitrogen depends on its C/N ratio (White, 1997). The decomposition (mineralization) of compounds with C/N ratio as high as 60/1 or 80/1 results soil nitrogen deficiency. This is because microbes (bacteria) have C/N ratios 4/1 or 6/1 and fungi have ratio from 10/1 to 12/1 and some mineralized nitrogen must be

used by microbial community to balance their needs for new microbial biomass (Tate, 1995; Wong et al., 1998).

In such soil the growth of microorganisms will be reduced by nitrogen limitation which directly effects the productivity of plant materials. The data from different field showed when C/N ratio is  $< 25/1$  the soil immobilization is not a problem (does not limit) for plant community, which shows the existence of soil net nitrogen mineralization (Tate, 1995; Trinsoutrot et al., 2000).

### **1.2.1.1 Environmental Influences on Nitrogen Mineralization /Immobilization**

Nitrogen mineralization and immobilization are basic to microbial life and they can occur in any environmental condition where microbial growth is possible. Soil physical and chemical properties effect the rate of these two nitrogen conversions. The primary ecosystem properties, which usually have impact on these processes, are nitrogen concentration, soil moisture, pH, and temperature. There is an optimum level of these conditions in which the biological activity tends to increase to a maximum level.

With no other limitation nitrogen mineralization and nitrogen immobilization increases with increasing water content. On the other hand when soils become saturated oxygen diffusion becomes a limiting factor for microbial respiration. Nitrogen turnover can continue under anaerobic conditions at a reduced rate because the metabolic rate in anaerobic organisms is low compared with those, which occur in aerobic condition (Flowers and Arnold, 1983). Immobilization is rarer in anaerobic condition because the efficiency of carbon metabolism is low. Optimum moisture content for aerobic transformation of nitrogen is about 60-90% of the saturation. Optimum soil pH for these activity is neutral and optimum temperature is 40-60°C (Tate, 1995).

### **1.2.2 Nitrification**

Nitrification is the process, which biologically oxidizes the reduced form of nitrogen such as (i.e.) ammonium to nitrate. This reaction is divided into two steps and is

commonly carried out by two groups of autotrophic organisms. The first step conducted by  $\text{NH}_4^+$  oxidizers or primary nitrifiers, whereas the second step is carried out by  $\text{NO}_2^-$  oxidizers or secondary nitrifiers. These two groups are together addressed as Nitrobacteria. The first group includes bacteria belonging to the genus of *nitrosomonas* and the second group includes bacteria belonging to the genus *nitrobacter*.

First step  $2\text{NH}_4^+ + 3\text{O}_2 \rightarrow 2\text{NO}_2^- + 4\text{H}^+ + 2\text{H}_2\text{O};$

Second step  $2\text{NO}_2^- + \text{O}_2 \rightarrow 2\text{NO}_3^-.$

Hydrogen ions are produced by ammonium oxidation and molecular oxygen is used in these processes. These reactions convert the most reduced form (-3) of the nitrogen to the most oxidized form (+5). Nitrification is an obligatory aerobic process, which results acidification of the soil environment.

The primary source of ammonium in most soils is ammonification. There are also external sources such as sewage or other waste substances and nitrogenous fertilizers, which is applied to cropped land (Tate, 1995; White, 1997).

In the first step ammonium oxidizes to nitrite, which usually does not accumulate under normal situation in soils. The nitrite is usually oxidized to nitrate very rapidly (Tate 1995; Wrage et al., 2001).

Nitrification can be carried out both by autotrophic nitrifiers and heterotrophic nitrifiers. In heterotrophic nitrification nitrifiers use organic carbon as a source of C and energy (Wrage et al., 2001). Nitrate can be absorbed by plants. Alternatively since nitrite and nitrate are mobile they can leached and eventually lead to contaminated and eutrophication of lakes and rivers or become substrate for denitrification (Prosser, 1986).



### **1.2.2.1 Environmental Properties Affecting Nitrification**

Despite the mineralization process, which can be undertaken by variety of microbes, nitrification is carried out by a limited number of bacteria species (Tate, 1995). The factors which affects nitrification are:

- 1- Temperature
- 2-  $\text{NH}_4$  availability
- 3- Oxygen
- 4- pH
- 5- Soil moisture
- 6- Heavy metal and salinity

#### **-Temperature**

Ammonium oxidation to nitrite can occur at temperatures from about freezing point to 65°C, while the temperature range for the second step which is oxidation from nitrite to nitrate is from freezing to about 30-35°C and it is completely limited at 40°C. On the other hand it can be seen that nitrification occurs in tropical and subtropical soils which have higher temperatures. In these areas presumably some adaptation enables the process to continue (Paul and Clark, 1996; Addiscott, 1983; Sierra, 2001).

#### **- $\text{NH}_4^+$ availability (Substrate availability)**

Most soils contain an amount of nitrate and ammonium with rarely detectable nitrite levels. Nitrite oxidizers are generally controlled by the presence of nitrite, which is indirectly determined by ammonium availability.

The nitrification rate depends on the population density of nitrifier bacteria, which are able to use ammonium and nitrite efficiently. Therefore nitrate production may increase proportionally to ammonium concentration in the soil sample. On the other hand toxic concentrations of ammonium and nitrite can inhibit the process (Abbasi et al., 2001).

## **-Oxygen**

Nitrification can occur only in aerobic conditions. Molecular oxygen is needed in both steps of this process. Therefore any soil parameter which can have effect on oxygen diffusion has indirect effect on nitrification. On the other hand in some waterlogged soil such as rice plants the movement of air through the plant into the rhizosphere can help the process to occur (Paul and Clark, 1996).

## **-pH**

pH effects the both the number of the nitrifier and nitrification rate. They reduce at below pH 6.0 and are limited below pH 5.0. The optimum pH for this process is between 6.6 and 8.0. On the other hand nitrification does occur in forest soils. It is probably because of:

- a) The variation of microsite soil pH.
- b) Possible occurrence of nonculturable, acidophilic, autotrophic nitrifier in these soils.
- c) Probable occurrence of some heterotrophic nitrifier population
- d) Adaptation of some nitrifiers to the growth condition in these soils (Tate, 1995).

Nitrite is oxidized to nitrate very rapidly. Under alkaline condition where the pH rises above 8.0~8.5, nitrite may accumulate. This is because ammonia is toxic to *nitrobacter*. There is equilibrium between ammonium and ammonia in soils. In this equation ammonium gain advantage in neutral and acid soil while ammonia is predominated in alkaline soil condition. Therefore under alkaline condition ammonia is present and toxic to *nitrobacter* (Tate, 1995).



## **-Moisture**

Moisture is an important factor on soil microbial community since they are essentially aquatic. The nitrifier community also needs moisture both for growth and activity. Flowers and O'Callaghan, (1983) used different moisture content in nitrification incubation and found out the nitrification rates increases with increase of moisture content ( $-1.5\text{MPa}$  to  $-8.0\text{KPa}$  water potential). In extremely low water content or permanent wilting point the nitrification is limited. But as water increase nitrifier start growing and activity.

At the other extreme when soils become saturated, molecular oxygen availability and its diffusion rate in water becomes the limitation of nitrification.

## **-Heavy metals and salinity**

Contaminated soils where metal concentrations exceed their threshold values have significant impact on nitrification rate. The effect increases with metal concentration. These metals could be a variety of elements such as silver, cadmium, chromium, nickel, cobalt, copper, zinc and selenium etc. (Tate, 1995). The total metal content of soil is not sufficient to explain the variation in nitrification rates. This could be due to combination of direct and indirect effects. Nitrification could be influenced by total metal but the bioavailability of total soil metals are affected by soil properties determining speciation, thus soil properties may affect nitrification directly or indirectly through effect on metal speciation (Kostov and Van Cleemput, 2001).

Nitrification can also be affected directly by increasing salinity. Inhibition of the nitrification rate is different with different soil types and salts (Badia, 2000). There is a potential for soil microbial population to adapt to the toxicity of both heavy metals and salinity in soils (Tate, 1995).

### 1.2.3 Nitrogen Fixation

Nitrogen fixation is a major input to the soil nitrogen cycling. In this phenomenon the nitrogen atom is reduced from its (0) oxidation state molecular form ( $N_2$ ) to its most reduced form ( $NH_4^+$ ) by the following reaction:



There is an inevitable loss of nitrogen from all soils both in biological and abiological processes. Therefore some external supply of nitrogen is always required in terrestrial sites to maintain and support long term productivity. Otherwise soil nitrogen reserves slowly decline until vegetation productivity is reduced or even stopped. Soil nitrogen fixation can improve development of reclamation and management in damaged or mismanaged soils because the fixed organic nitrogen is mineralized later and helps to improve nitrogen cycle in this kind of soils.

Nitrogen fixation is catalyzed by a number of bacteria, that is no fungi, plants, or animals have this capacity. This bacteria which are called diazotrophs are either free living among the general soil microbial population or in symbiotic association (i.e., Rhizobium- legume or Actinorhizal Symbioses )

Symbiotic bacteria, which are associated with legume cultivation usually, reduce dinitrogen to  $NH_4$ , and this activity is catalyzed by an enzyme called dinitrogenase (White, 1997).

### 1.2.4 Volatilization

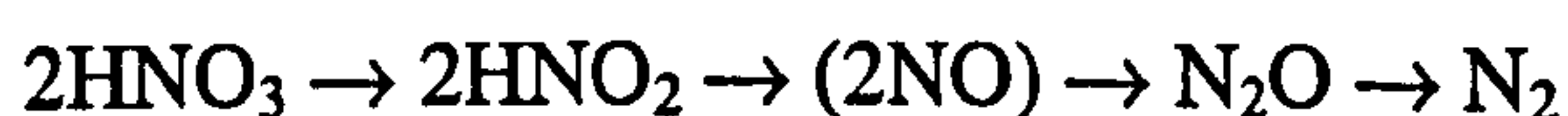
Ammonia volatilization can be a major route for nitrogen loss from the soil. For soils below pH 7.0 nitrogen volatilization is a minor problem, but in alkaline pHs the equilibrium between ammonia and ammonium changes to ammonia predominance (Roelcke et al., 1996).



### 1.2.5 Denitrification

Denitrification is a part of nitrogen cycle which is the stepwise reduction of different oxide forms ( $\text{NO}_2^-$  and  $\text{NO}_3^-$ ) of nitrogen to nitrous oxide and or dinitrogen. This return of nitrogen to the atmosphere is catalyzed by variety of soil bacteria, which can reduce nitrogen oxides in the absence of free oxygen. These nitrogen oxides behave as terminal electron acceptors for a bacterial respiration. Therefore the primary factors for the denitrification are the availability of energy source and nitrate and the absence of molecular oxygen in the micro-environment of the bacteria.

During biological denitrification nitrate is transformed to dinitrogen through series of reaction:



Nitric oxide is put in brackets because it is not usually detected as a free intermediate. The microorganisms, which can catalyze this process, are usually members of the genera *Pseudomonas*, *Alcaligenes* or many others (Tate, 1995; Paul and Clark, 1996).

The denitrification might be carried out by non biological reaction which is called chemodenitrification. This reaction is performed by chemical decomposition of intermediates from the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_2^-$  or of  $\text{NO}_2^-$  itself with organic (e.g. amines) or inorganic (e.g. Fe or Cu) compound (Wrage et al., 2001).

### 1.3 Phosphorus (P)

Phosphorus is defined as one of the macronutrients which is in the form of phosphate in the soil. Quite a substantial amount of phosphorus is associated with the soil organic matter. For most mineral soils apatites are believed to be the primary phosphate containing mineral from which the other P containing soil fractions are derived. Three main phosphate fractions are important from the viewpoint of plant nutrition:

- 1- Phosphate in soil solution.
- 2- Phosphate in labile form.
- 3- Phosphate in non-labile fraction.

The first fraction is clearly defined and is the phosphate dissolved in the soil solution. The second fraction is the solid phosphate, which is held on surfaces so that it is in rapid equilibrium with soil solution phosphate. The third fraction is the insoluble phosphate, which can be released only very slowly into the liable pool. Apatite, Fe, Al phosphate and organic soil P are considered as slow release soil-P (Mengel and Kirkby, 1978).

The amount of phosphate present in the soil solution is very low (0.3-3mg/l) in comparison with adsorbed phosphate ( $10^2$ - $10^3$  time higher). The most important P-containing ions in soil solution are  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$ . The ratio of these two species in soil solution is pH dependent. In high  $\text{H}^+$  concentration more protonated form is dominant. At a pH 5,  $\text{HPO}_4^{2-}$  is almost absent whereas at pH 7,  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$  are present in fairly equal proportions. The phosphate present in the soil solution can be absorbed by plant roots and it is replenished by phosphate, which diffuses from adsorbing surfaces (Mengel and Kirkby, 1978).

In highly weathered soils where the effects of the Fe and Al are predominant the phosphate is mainly in the form of insoluble salts of Fe and Al, which the solubility increase with pH. While in the alkaline and calcareous soils insoluble Ca phosphate is exist which the solubility increase with decreasing of pH.



Phosphate mineralization and assimilation are catalyzed by the general soil biological community. The quantity of water soluble phosphate is controlled not only by biological mineralization process but also by dissolution rates from the soil mineral fraction. In the phosphate cycle there is no change in oxidation state and there are no gaseous compounds to the transformation (Tate, 1995).

Plants suffering from P deficiency are small with a limited root systems and thin stems. The formation of fruit and seed is especially depressed in plant suffering from P deficiency (Mengel and Kirkby, 1978).

In order to maintain intensive grassland vegetation it is important to keep the balance between all three major nutrients N, P and K. Otherwise there might be limited response to nitrogen and poor grass growth. If P and K level are adequate, nitrogen can be used to manipulate both the rate and the amount of grass growth because the grass growth can be more with more nitrogen.

## **1.4 Aim of the Study**

Human activities can release large amounts of chromium into the soil and have adverse effects for both human health and plant system. Various factors are required for the establishment and maintenance of vegetation as a part of the reconstruction of contaminated industrial land. These factors are light, temperature, CO<sub>2</sub>, water, O<sub>2</sub> and adequate amounts of major nutrients.

A former industrial site, which is contaminated with chromium, is intended to be studied. The poor vegetation and poor establishment of trees in the central part of the site on the spoil heap of the area led to the investigation of this area. The site is called Summerford, and is situated at the end of the Summerford Road, just off Windsor Road in Falkirk. The primary investigation showed pools of water with a yellow crystalline deposit with a pH of 9 and 60mg/l of soluble chromium going to the road and the storm drain carrying some sediment from the area.

The understanding of the total nutrient conditions in plant and soil of this industrial contaminated land is important for better reconstruction. The probable toxicity of chromium on the plant growth and other factors such as different major nutrient availability and limitation of the soil i.e. nitrogen and phosphorus are underlying aims to be investigated in order to provide a good vegetation cover on the soils.

Chromium toxicity may itself have a direct toxic or inhibitory effect on plant growth. It might also have effect on nutrition turnover in the soil system. The work would contain a survey of plant and soil nutrition and heavy metals and the effects of different fertilizers on the plant growth. Since the nitrogen is one of the major mineral nutrients required for growth and development of plants, it is intended to assess some aspects of nitrogen turn over such as mineralization, nitrification and volatilization in this contaminated site.

In order to better understand the nutrient availability of the growth medium, an analysis of leaf and plant tissue is necessary. Plant analysis helps soil analysis to give a better answers to fertilizer needs in agriculture, the heavy metals and contaminated materials for national and international health purposes and regulations.

As part of the project an analytical research on plant-P analysis is intended to be carried out. This analytical study deals with problems with phosphate analysis in digest solutions and evaluation of different digestion methods for phosphate-P determination in vegetation samples.

A study has been carried out by a student in this department. He tried to use a single digestion method for N, P and K determination. He obtained higher recovery of plant-P in certified plant material. In order to complete the his research work, several digestion methods including the Kjeldahl method is going to be tested in the evaluation of plant-P analysis. In this experiment certified and other plant materials will be used and different temperatures and heating systems will be tested.



## **CHAPTER 2**

### **METHODS**

This chapter is a brief description of apparatus, reagents and their preparation, procedures and calculation of the results.

#### **2.1 ROUTINE METHODS**

##### **2.1.1 Soil Sample Preparation**

The soil samples were taken to the laboratory. Each soil sample was divided to two parts, one part was stored at 4° C in the cold room and the other part was spread out on clean plastic sheet for drying at laboratory temperature for 4 days. The air dried samples were ground and sieved through a 2mm sieve.

##### **2.1.2 Plant Sample Preparation**

The plant samples were taken to the laboratory and contaminating material was removed. They were then weighed, and put into the oven at 80° C for 48 hours and weighed again as yield of grass in a square metre.

The samples were ground (1mm), with Glen Creston Mill 14680, and stored in desiccator, until analysis.

##### **2.1.3 Measurement of Soil pH**

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

Soil pH was determined in a 1:2.5 soil: deionized water suspensions by a combined glass-reference electrode and Mettler Delta 320 pH meter according to MAFF/ADAS (1986).

Duplicate suspensions were prepared by weighing 20 g sample of each soil into 120 ml screw cap glass bottles and 50 ml deionized water was added to each bottle. The caps were closed tightly and the bottles were shaken for half an hour on an end-over shaker.

The pH meter was standardised with buffer solutions of pH 7.0 and pH 4.0. The suspensions were mixed thoroughly by shaking briefly. The electrode was then immersed in the bottle and the soil suspension stirred by swirling the electrode slightly. The pH was recorded.

#### **2.1.4 Measurement of Soil Conductivity**

Soil conductivity was determined in a 1:2.5 soil: deionized water by a Jenway 4070 conductivity meter.

Duplicate suspensions were prepared weighing 20g samples of each soil into 120ml screw cap glass bottles and 50ml deionized water was added to each bottle. The caps were closed tightly and the bottles were shaken for half an hour on an end-over shaker. The soil suspensions were filtered through a Whatman No.1 filter paper into clean universal bottles pouring as much of the solid as possible into the filter paper along with the solution. This helps produce a clearer filtrate. Any filtrate that was not clear was refiltered through the original filter paper plus.

The Electrical Conductivity meter was switched on and the conductivity cell probe was immersed in the solution so that the measuring chamber was completely filled and solution was above the breather holes in the stem of the probe. The conductivity electrode was swirled in the filtrate solution and the conductivity value was read.

#### **2.1.5 Determination of Moisture Content of Soils**

Silica basins were washed and dried in an oven at 105°C for 24 hours, cooled in a desiccator and weighed with a four figure balance. They then were put in the oven and weighed again until their weight became constant.

A suitable weight (about 10g) of air dried soil sample, in duplicate, was placed in each basin and weighed. The soil and basin were put in an oven at 105° C for 24

hours. They then were taken from the oven and cooled in a desiccator and reweighed. The percentage of moisture content of soil was determined on oven dried basis as follows:

$$\% \text{moisture content} = \frac{\text{weight of air dried soil} - \text{weight of oven dried soil}}{\text{weight of oven dried soil}} \times 100$$

### **2.1.6 Determination of Moisture Content of Plants**

Silica basins were washed and dried in an oven at 105°C for 24 hours, cooled in a desiccator and weighed with a four figure balance. They then were put in the oven and weighed again until their weight became constant.

A suitable weight (about 2 g) of air dried plant sample in duplicate was placed in each basin and weighed. The plant and basin were put in an oven at 80° C for 24 hours. They then were taken from the oven and cooled in a desiccator and reweighed. The percentage of moisture content of plant was determined on oven dried basis as follow:

$$\% \text{ moisture content} = \frac{\text{weight of air dried plant} - \text{weight of oven dried plant}}{\text{weight of oven dried plant}} \times 100$$

### **2.1.7 Determination of Soil Moisture Content at -0.5 bar**

Determination of moisture content of soils at -0.5 bar was done with pressure plate apparatus (Manifold # 750 Series, Moisture Equipment Company, Santa Barbara, California, USA).

Three replicates of each soil were placed on the plate surrounded with a rubber ring, and flooded with water. They were covered with plastic sheet to avoid any evaporation of water and allowed to saturate for 24 hours. They were then placed in the pressure vessel, which was adjusted to -0.5 bar using a nitrogen gas cylinder. The pressure vessel containing the soil samples was allowed to equilibrate for 72 hours until water loss ceased. The soils were weighed immediately after removal



from the pressure plate apparatus and their percentage moisture contents were determined on oven dry basis (section 2.1.5).

### **2.1.8 Determination of Loss On Ignition (LOI) in Soils**

After soil moisture determination, the silica basins containing oven dried soil samples were placed in the electric muffle furnace at 500° C for 5 hours. They were left to cool and then transferred to an oven at 105° C for 1 hour. They then were cooled in a desiccator and were weighed again. Their Loss On Ignition (LOI) contents were calculated as follow:

$$\%LOI = \frac{\text{weight of oven dried soil} - \text{weight of muffle furnace dried soil}}{\text{weight of muffle furnace dried soil}} \times 100$$

### **2.1.9 Measurement of Organic Matter (Dichromate Method)**

Soil organic matter was completely oxidised by gently boiling the soil samples with a mixture of potassium dichromate solution, sulphuric and orthophosphoric acids. The residual of dichromate was determined by titrating with ferrous sulphate solution (MAFF/ADAS, 1986).

#### **2.1.9.1 Reagents**

##### **- Barium diphenylamine sulphonate**

0.2g of (AR) barium diphenylamine sulphonate (this substance is toxic) was dissolved in approximately 150ml of warm water. Then 20g of barium chloride (AR) was added to the solution, warmed to dissolved, cooled and then diluted to 200ml.



**- Ferrous sulphate (approximately 0.4 M)**

5 ml of concentrated sulphuric acid was added to about 1.5 litre of deionized water. 320 g of (AR) ammonium ferrous sulphate was dissolved in this solution. It was then made up to 2 litre with deionized water.

**- Potassium dichromate (66.7 mM)**

Potassium dichromate powder was dried at 102° C for overnight and then it was cooled in desiccator. 39.23g of the dried salt was dissolved in approximately 700ml of deionized water. 800ml of concentrated sulphuric acid was added very slowly with stirring, and cooled. Then 400ml of concentrated orthophosphoric acid was added, stirred to dissolve the chromic acid, cooled and diluted to 2 litre with deionized water.

### **2.1.9.2 Procedure**

Air dried soil which previously ground to pass through a 2mm sieve, was ground again to pass a 0.5mm sieve and mixed well. 0.5g of each soil was weighed in duplicate and put into tall digester block tubes. 20ml of potassium dichromate solution was added to each and the tubes in block were heated in the block at 130-135°C for 2 hours. They then were cooled, removed from the block and the contents transferred to 500ml conical flask with washing. 100ml of deionized water and 2ml of barium diphenylamine sulphonate reagent were added to each flask. It was then titrated with approximately 0.4M ferrous sulphate solution. When a purple colour appeared, just before the end point, titration was continued by dropwise addition until the colour changed to bright green. The amount of used ferrous sulphate reagent was recorded (yml).

Blank digestions were carried out with no soil sample added to the tubes.

Two replicates of this experiment were carried out for each soil.

### 2.1.9.3 Standardisation of Approximately 0.4 M Ferrous Sulphate

20ml of potassium dichromate was pipetted into a 500ml conical flask. 100ml of deionized water and 2ml of barium diphenylamine sulphonate were added. It was then titrated with approximately 0.4M ferrous sulphate solution. When a purple colour appeared, just before the end point, titration was continued by dropwise addition until the colour changed to bright green. The volume of ferrous sulphate was recorded (x ml). Standardisation was carried out before the examination of any samples.

$$\text{Standardisation factor, } f, \text{ of 0.4 M ferrous sulphate} = \frac{20}{x}$$

### 2.1.9.4 Calculation

The organic matter was calculated as g/kg of soil from the equation below:

$$\text{g/kg of soil organic matter} = \frac{1.2 \times 1.724 \times (V - fy)}{W}$$

Where:

- It was assumed: 1ml of 66.7 mM potassium dichromate = 1.2mg carbon.
- Soil organic matter = carbon  $\times$  1.724
- W: Weight of soil.
- V: The volume of potassium dichromate used.
- f: Standardisation factor.
- y: Volume of ferrous sulphate used for titration of samples.
- x: Volume of ferrous sulphate used for titration of 20ml potassium dichromate.

## **2.2 EXTRACTION METHODS**

### **2.2.1 Extraction of Inorganic Nitrogen ( $\text{NH}_4^+\text{-N}$ , $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ ) from Soil**

#### **2.2.1.1 Washing the Filter Paper**

##### **- Preparation of 0.5 M sulphuric acid solution for washing filter paper**

Working in the fume cupboard 27.77ml of grade concentrated sulphuric acid, measured by measuring cylinder, was added to about 900ml deionized water in a 1 litre volumetric flask. After cooling it made up to 1 litre with deionized water.

##### **- Washing procedure**

Washing the filter paper was done according to Shah (1988). Each filter paper was folded separately into a clean and dry plastic funnel. 50ml 0.5M sulphuric acid was filtered through each filter paper in two portions of 25ml each. Then the filter paper was washed 5 times with deionized water to remove any acid left in the filter paper. Care was taken to make sure that filter papers were made acid free. Litmus test paper was used for this purpose. The funnels containing filter paper were then allowed to dry in a 70° C oven for 4 hours.

#### **2.2.1.2 Purification of 0.5 M Potassium Sulphate**

##### **- 1M Potassium Hydroxide**

5.61g of (AR) potassium hydroxide was dissolved in 100ml deionized water

##### **- 0.5 M sulphuric acid**

As described in section 2.2.1.1.



## **- Purification**

This solution was prepared according to Khan (1987). 87.125g of potassium sulphate was dissolved in about 800ml deionized water and made up to 1 litre. The pH of solution was adjusted to 11 with 1M potassium hydroxide in order to convert any ammonium in the solution to ammonia. It was then made up to volume and boiled on a gas burner for 15 minutes to remove any ammonia gas. The solution was cooled and pH was adjusted to 5~5.5 with 0.5M sulphuric acid. Khan (1987) and Shah (1988) adjusted the solution to pH 6, but in our study it was decided to adjust the solution to slightly lower pH because the soils pH are above 8.3. It was then made up to volume by adding deionized water, because during boiling some water was evaporated from the solution.

### **2.2.1.3 Procedure of Soil Extraction**

An amount of fresh soil equivalent to 2.5g of oven dried of soil samples was weighed in duplicate into 100ml (3oz) glass screw cap bottles and 50ml of purified potassium sulphate solution was added with an automatic pipette to each. The glass bottles were capped with plastic tops and then shaken at 2°C in the cold room with a reciprocating shaker for 2 hours. The suspensions were filtered through washed Whatman No.2 filter papers. The extracts were collected in plastic bottles and stored in the cold room in 2°C.

Blanks:

50ml potassium sulphate was transferred into 100ml glass bottle, capped, shaken and filtered with the same manner on the soil samples.

### **2.2.1.4 The Storage of Extracts**

The filtered soil extracts were stored in a cold room at 2°C prior to analysis. Khan (1987) studied the extractable inorganic nitrogen and based on his experiments he



recommended that if analysis is not possible immediately, extracts can be stored at 2°C for two months.

## **2.2.2 Extraction of Chromium from Soil with Water**

### **2.2.2.1 Procedure**

An amount of fresh soil equivalent to 2.5g of oven dried soil samples were weighed in duplicate into 100ml (3oz) glass screw cap bottles and 50ml of degassed deionized water was added with an automatic pipette to each. The glass bottles were capped with plastic tops and then shaken at 2°C in the cold room with a reciprocating shaker for 2 hours. The suspensions were filtered through washed Whatman No.2 filter papers. The filtrates were refiltered through 0.2µm filter again, to be ready for analysing by Ion Exchange Chromatography for chromate.

Blanks were carried out with no soil sample was added to the bottles.

These filtrates can also be analysed for water extractable nitrate, nitrite, phosphate, chloride and sulphate.

## **2.2.3 Extraction of Phosphorus from Soil**

Phosphorus was extracted from soil at 20°C with a sodium bicarbonate solution of pH 8.5. The concentration of blue complex produced by reduction with ascorbic acid, of the phosphomolybdate formed when acid ammonium molybdate reacts with phosphate can be measured spectrophotometrically at 880nm (MAFF/ADAS, 1986) for analysis of phosphate.

### **2.2.3.1 Reagents**

#### **- Sodium hydroxide solution (50 % m/m)**

10g of sodium hydroxide was dissolved in 10ml of deionized water in the fume cupboard.

#### **- Polyacrylamide solution (0.05 m/v)**

0.5g of polyacrylamide was dissolved in approximately 500ml deionized water. Its dissolution needs several hours stirring. After the dissolution of the polymer it was made up to 1 litre with deionized water.

#### **- Sodium bicarbonate reagent (0.5 M)**

84g sodium bicarbonate was weighed and dissolved in about 1500ml deionized water. 10ml of 0.05% m/v polyacrylamide solution was added to the solution and then it was made up to 2 litre with deionized water. The pH of solution was adjusted to 8.5 at 20°C with 50% m/m sodium hydroxide solution. Its pH was also checked on the day of use.

### **2.2.3.2 Procedure**

An amount of fresh soil equivalent of 2.5g oven dried soil samples was weighed into duplicate 100ml (3oz) glass screw cap bottles and 50ml of sodium bicarbonate reagent of pH 8.5 was added to each with an automatic pipette. The bottles were capped and shaken on an end-over shaker for 30 minutes at 20°C. The suspensions filtered through Whatman No. 2 filter paper. The filtrates were retained for the determination of phosphorus.

Blanks were carried out with no soil sample added to the bottles.

### **2.2.4 Extraction of Potassium from Soil**

Potassium was extracted from soil with M ammonium nitrate (MAFF/ADAS, 1986). The concentration of potassium in the extract was measured by flame photometer as described in section 2.4.9.

### **2.2.4.1 Reagents**

#### **- Ammonium nitrate (M)**

400g of (AR) ammonium nitrate was dissolved in water and diluted to 5 litre.

### **2.2.4.2 Procedure**

An amount of fresh soil equivalent to 5g oven dried soil samples was weighed with a two decimal place balance in duplicate into 100ml (3oz) glass screw cap bottles and 50ml of M ammonium nitrate was added to each with an automatic pipette. The bottles were capped and shaken on an end-over shaker for 30 minutes at room temperature. The suspensions were filtered through Whatman filter paper No.40. The filtrates were retained for determination of potassium. Magnesium can also be measured in this extract.

Blanks were carried out with no soil sample added to the bottles.

### **2.2.5 Extraction of Magnesium from Soil**

Reagent and extraction procedure is the same as the method of extraction of potassium from soils, which is described in section 2.2.4.

## **2.3 DIGESTION METHODS**

### **2.3.1. Plant and Soil Digestion with Perchloric Acid**

In order to provide a useful approach of total nutrients such as phosphate, potassium and metals plants and soils were digested with concentrated perchloric acid.

#### **2.3.1.1 Reagents**

##### **- Concentrated nitric acid (AR)**



- Concentrated perchloric acid (AR)

### **2.3.1.2 Procedure**

An amount of approximately 0.2g dried 1mm plant material or soil was weighed out accurately (four decimal places) into a tall form Pyrex beaker. 10ml concentrated nitric acid was added. The beakers were covered with a watch glass and allowed to stand for overnight predigestion. The samples were then heated on a hotplate at approximately 100°C until brown fumes of nitrogen dioxide (NO<sub>2</sub>) were no longer evolved. The temperature was carefully increased until the acid was boiling gently. After 3 hours the watch glasses were removed and heating continued until the volume of each digest solution was reduced to approximately 5ml. The samples were removed from the hotplate and allowed to cool completely. Three ml concentrated perchloric acid were added and the digests were heated to just below the boiling point (180°C) to evaporate the remaining nitric acid. When dense white fume of perchloric acid appeared, the beakers were covered with a watchglass and the heating were increased to a very gentle boiling (180°C), (taking particular care that the digest did not boil dry), for two hours. The digests were cooled and 10-15ml deionized water added washing the watchglasses into the beakers. The digests were filtered through Whatman No. 50 filter papers into 50ml volumetric flasks. The beakers were rinsed with at least three 10ml aliquots of deionized water, and filtered as separate aliquots into the volumetric flask. Finally the volume was made up to 50ml with deionized water and the solution mixed well.

### **2.3.2 Plant Digestion with Kjeldahl Method for Total Nitrogen**

Plant material was digested according to Bremner and Mulvaney (1982).

#### **2.3.2.1 Reagents**

- Kjeltabs



Kjeltabs, which were used in this experiment, were purchased from Thompson and Capper Ltd. 3 Goddard Rd. Astmoos Industrial Estate, Runcorn, Cheshire WA7 1PH England. Each tablet contains 5g of the mixture (100 parts  $K_2SO_4$  plus 6 parts  $CuSO_4 \cdot 5 H_2O$  and 1 part selenium).

Selenium is a toxic substance and care should be taken while working with it in the fume cupboard.

### **- Sulphuric acid**

Concentrated (AR) sulphuric acid was used.

### **2.3.2.2 Procedure**

An amount of approximately 0.2g dried plant material was weighed out accurately (four decimal places) into an aluminium foil weighting boat. The plant sample was placed in the bottom of the 30cm long digestion tube using long forceps. The weighting boat was weighed again to obtain the exact weight of sample digest in case a small amount of plant sample is remained in it. 2.5g catalyst salt mixture (half of a tablet) was put in each tube and 5ml concentrated sulphuric acid was added. The samples were left overnight for predigestion and then placed in a cold digestion block. The temperature was increased gradually until the digest cleared. It was then adjusted to 375°C. The heating was continued for another one hour.

The digestion tubes were cooled and removed from the digestion block. About 10ml deionized water was added to each tube, mixed and cooled again. The digests were filtered quantitatively through Whatman No. 50 filter paper into a 100ml volumetric flask. The tubes were washed 3 times with deionized water, which was added to filter paper.

Finally the volumetric flasks were made up to volume with deionized water, mixed well and stored to be analysed.

### **2.3.3 Soil Digestion with Kjeldahl Method for Total Nitrogen**

The procedure was the same as the method, which is described in 2.3.2 with the following modification:

- The weight of the soil samples was 0.5g.
- After clearing the digests, heating continued at 375°C for three hours, to obtain complete digestion.

## **2.4 ANALYSIS METHODS**

### **2.4.1 Determination of Total Heavy Metals in Soil and Plant Digests**

Digestion solutions of soils or plants which were digested with concentrated perchloric acid (section 2.3.1) were analysed for determination of total chromium, zinc, copper, nickel, lead and cadmium by Atomic Absorption Spectrophotometer Perkin-Elmer Model 1100.

#### **2.4.1.1 Reagents**

##### **- Working standard solutions**

The working standard solutions were prepared from 1000mg/l stock solution supplied by BDH Spectrosol as follows:

Cadmium	0, 0.4, 0.8, 1.2, 1.6 and 2mg/l.
Zinc	0, 0.2, 0.4, 0.6, 0.8 and 1mg/l.
Nickel	0, 0.4, 0.8, 1.2, 1.6 and 2mg/l.
Lead	0, 4, 8, 12, 16 and 20mg/l.
Chromium	0, 1, 2, 3, 4 and 5mg/l.
Copper	0, 1, 2, 3, 4 and 5mg/l.

Each standard solution contained 5% perchloric acid.

2.4.1.2 Procedure

For analysis of total of the each metal Atomic Absorption was adjusted with working standard solutions of the metal and an appropriate linear graph for each metal was constructed.

2.4.2 Determination of Available Magnesium

Available magnesium was determined in M ammonium nitrate extracts using Atomic Absorption Spectrometry. Phosphate reacts with magnesium in the solution and produces magnesium hydrogen phosphate, which inhibits the formation of free magnesium atom in the flame. Lanthanum or strontium is usually added to react with the phosphate instead of magnesium in the solution.

After adding the lanthanum chloride to the soil extracts containing chromate, lanthanum precipitated as lanthanum chromate.

Since the solubility of strontium chromate is higher than lanthanum chromate (Table: 2.1), strontium chloride was chosen to react with phosphate ion in the extract solution, instead of lanthanum chloride.

This procedure is also suggested for determination of calcium by Atomic Absorption.

Type of salt	Solubility g/l	
	15°C	25°C
La <sub>2</sub> (CrO <sub>4</sub> ) <sub>3</sub>	-	0.020
SrCrO <sub>4</sub>	0.88	0.91

Table 2.1: Solubility of La<sub>2</sub>(CrO<sub>4</sub>)<sub>3</sub> and SrCrO<sub>4</sub> salts ( Kafarov 1963)

2.4.2.1 Reagents

- Strontium chloride solution (10%)



10g of (AR) strontium chloride salt was dissolved in 100ml of deionized water.

**- Magnesium stock solution (1000g/l)**

Magnesium stock solution was supplied by BDH Spectrosol.

**- Working standard solutions (0, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/l Mg)**

Appropriate amounts of magnesium standard solution were transferred to six different 25ml volumetric flasks. 2.5ml of 10% strontium chloride solution was added to each and they then made to 25ml with M ammonium nitrate solution.

### **2.4.2.2 Procedure**

20ml of each soil extract was transferred into a 25ml volumetric flask by pipette. 2.5ml of 10% strontium chloride solution was added to each and made up to 25ml with deionized water. Samples, blanks and standards were analysed using Atomic Absorption Spectrophotometer Perkin-Elmer Model 1100.

The extracts were diluted if necessary provided that the final concentration of strontium chloride in solution would be 1%.

### **2.4.3 Determination of Available Ammonium in Soil Extracts**

Ammonium was determined using automated colorimeter on a Technicon Auto Analyzer II system. The procedure was an indophenol green method using a complexing reagent to prevent interference due to the precipitation of hydroxides in the reagent system. With the inclusion of sodium nitroprusside catalyst the sensitivity of the method is such that ammonium nitrogen can be determined in a range of 0-1mg/l (Brown 1973, Metwaly 1999).



### **2.4.3.1 Reagents**

Analar grade reagents and nitrogen free deionized water were used throughout the experiment. The reagents were prepared in the fume cupboard. The fan was switched off while actual weighing was in process but otherwise was kept running.

#### **- Alkaline phenol**

25g of sodium hydroxide was dissolved in about 900ml deionized water in a 1 litre dark glass bottle. Working in a fume cupboard very carefully 45g phenol was weighed into a 1 litre beaker and approximately 500ml sodium hydroxide solution was added and stirred with a glass rod to dissolve the phenol. The solution was returned to the bottle and the volume made to 1 litre and mixed gently. The solution was degassed in a ultrasonic bath for 10 minutes. The dark glass bottle must have plastic stopper not a glass one.

#### **- Complexing reagent**

50g potassium sodium tartrate and 50g sodium citrate were dissolved in approximately 900ml deionized water in a 1 litre bottle. Working in the fume cupboard 0.5g sodium nitroprusside was weighed carefully into a 100ml beaker, 50ml water was added and stirred gently using a magnetic stirrer. The resulting solution was added to the citrate tartrate solution, made to 1 litre and degassed in a ultrasonic bath for 10 minutes. 1ml 15% Brij-35 solution was added and mixed gently.

#### **- Sodium hydrochloride solution (approximately 0.5 %):**

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

#### **- Wash solution**

1ml of 15% Brij-35 was added to 1 litre of deionized water and mixed gently.

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

4.717g of dried ammonium sulphate was dissolved in deionized water and the volume made to 1 litre. The solution was stored at 2°C. Working standards were prepared by dilution of this solution.

**- Working standard solution (0 and 1 mg/l)**

0 and 1mg/l working standard solutions was made in 0.5M potassium sulphate in two 100ml volumetric flasks.

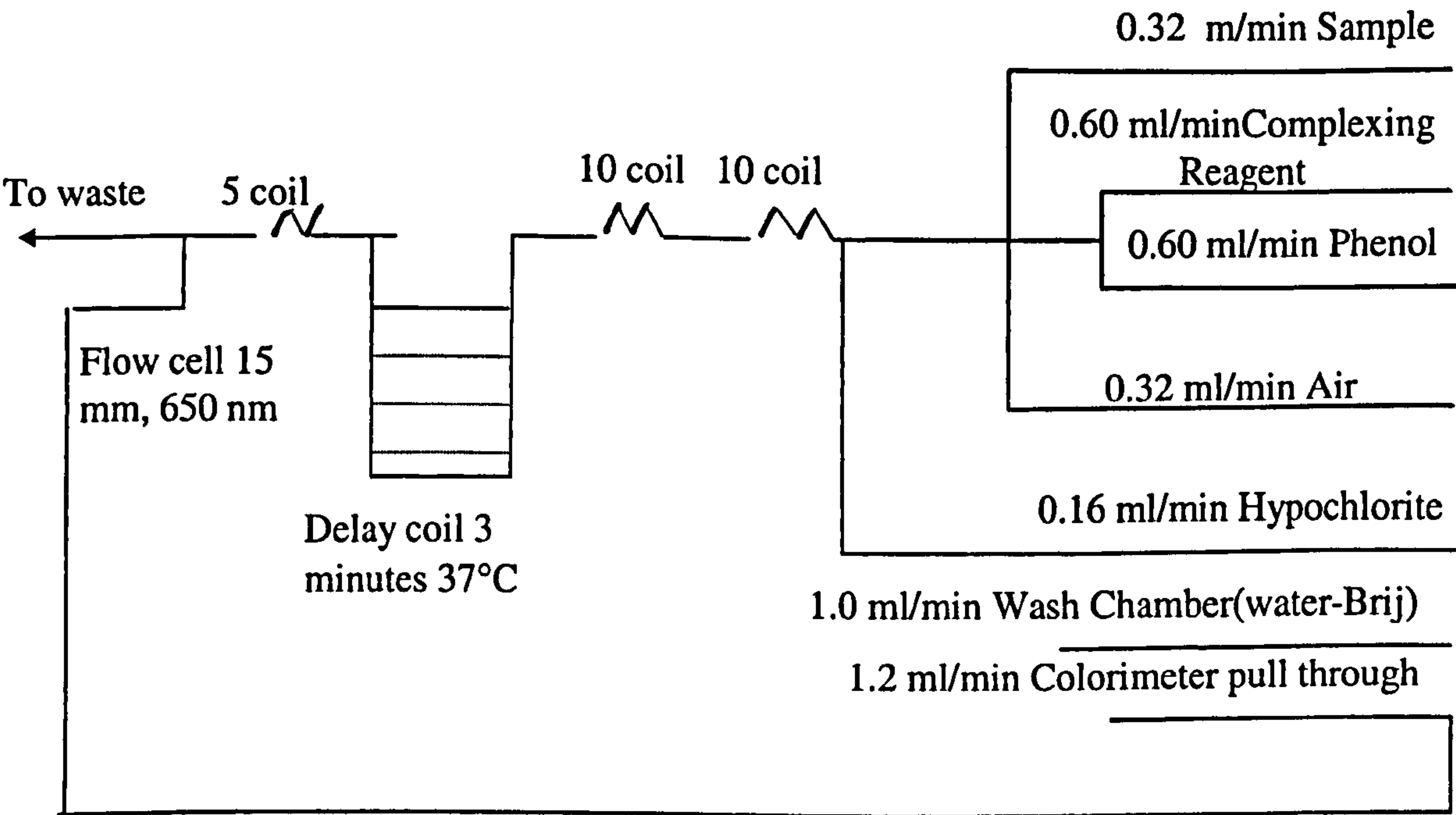


Figure 2.1: Auto Analyser manifold for determination  $\text{NH}_4\text{-N}$  in soil extract.

**- Apparatus**

The Technicon Auto Analyser II was used for the analysis of ammonium-nitrogen because its sensitivity, speed and ease of use. The Technicon Auto Analyser II system consisted of a sampler, proportioning pump, a water bath at constant temperature 37°C and colorimeter equipped with 650nm filters and phototube

CE34Q. 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 peak heights and calculation of results. The reagent bottles were also put in a separate water bath at a constant temperature of 25°C.

### **2.4.3.2 Procedure**

The filtered solutions were analysed using the manifold Figure 2.1 along with standard solutions, blanks and zeros. Air was cleaned from atmospheric ammonium by bubbling through 5% sulphuric or hydrochloric acid. The samples were run at the rate of 50 per hour and the colour was developed in the water bath at 37°C. The colour intensity was measured at 650nm. The calibration graph for ammonium is linear from 0-5mg/l ammonium-N.

### **2.4.4 Determination of Ammonium-N in Kjeldahl Digests of Soil and Plant for Total-N**

Soil and plant material was digested by the method of Bremner and Mulvaney (1982). During the digestion procedure plant and soils organic nitrogen is converted to ammonium and ammonium was measured with the method described in section 2.4.3 with the addition of a diluting-neutralising step before the main manifold colour formation. 20 fold dilution was carried out using sodium hydroxide solution.

#### **2.4.4.1 Reagents**

##### **- Working ammonium standard solutions (0 and 50 mg/l)**

0 and 5ml of 1000mg/l stock solution was added to solutions containing 5ml concentrated sulphuric acid and 2.5g Kjeltab in two 100ml volumetric flasks to make 0 and 50mg/l standard solution respectively.

#### **- Wash solution (5% Sulphuric acid)**

Working in fume cupboard 50ml concentrated sulphuric acid was added to 800ml of deionized water. It was made to 1 litre with deionized water after cooling.

#### **- Diluter-Neutralization solution**

3.6g sodium hydroxide was dissolved in 1 litre deionized water.

### **2.4.4.2 Procedure**

The digest solutions were analysed as described in section 2.4.3.2 in addition of using the manifold with a dilution step prior to colour formation (Figure: 2.2) along with standard solutions, blanks and zeros.





### **2.4.5.1 Reagents**

#### **- Buffer solution**

22.5g sodium tetraborate (AR) and 2.5g sodium hydroxide (AR) was dissolved in 900ml deionized water and the volume was made up to 1 litre with deionized water. Then the solution was degassed in an ultrasonic bath for 10 minutes.

#### **- Greiss reagent**

Working in fume cupboard carefully 100ml concentrated (AR) hydrochloric acid was added into approximately 800ml deionized water using a measuring cylinder. The solution was degassed. Working in a fume cupboard, 10g sulphanilamide (AR) and 0.5g N-1-naphthylenediamine dihydrochloride (AR) were weighed into a 1 litre beaker. 500ml of acid solution was added carefully and was stirred gently using a magnetic stirrer. It was returned to the bottle and the volume was made up to 1 litre with degassed deionized water. Then it was mixed gently. The solution was stored at 2°C in the cold room.

#### **- Wash chamber solution**

1ml of 15% Brij-35 was added to 1 litre of deionized water and mixed gently.

#### **- Water Brij**

1ml of 15% Brij-35 and 5 ml of 100mg/l chromate solution was added to deionized water and made up to 1 litre. This solution contains 0.5mg/l Cr(VI) and was used for chromium contaminated sites.

#### **- Nitrite nitrogen stock solution (1000 mg/l)**

Sodium nitrite was dried at 105°C for 1 hour and cooled in a desiccator. 4.926g of dried sodium nitrite was dissolved in deionized water and the volume was made up to 1 litre with deionized water. The solution was stored at 2°C.

**- Working nitrite nitrogen standard solutions (0 and 1 mg/l)**

Working nitrite nitrogen standard solutions were prepared by dilution of stock solution in 0.5M potassium sulphate.

**2.4.5.2 Procedure**

The Technicon Auto Analyzer II was used for the determination of nitrite nitrogen in 0.5M potassium sulphate soil extracts. Samples were run at the rate of 50 samples per hour and the colour was measured at 530 nm. Nitrite nitrogen has a linear calibration in the range of 0-1mg/l.

The schematic diagram Figure 2.3 shows the flow system for nitrite nitrogen.

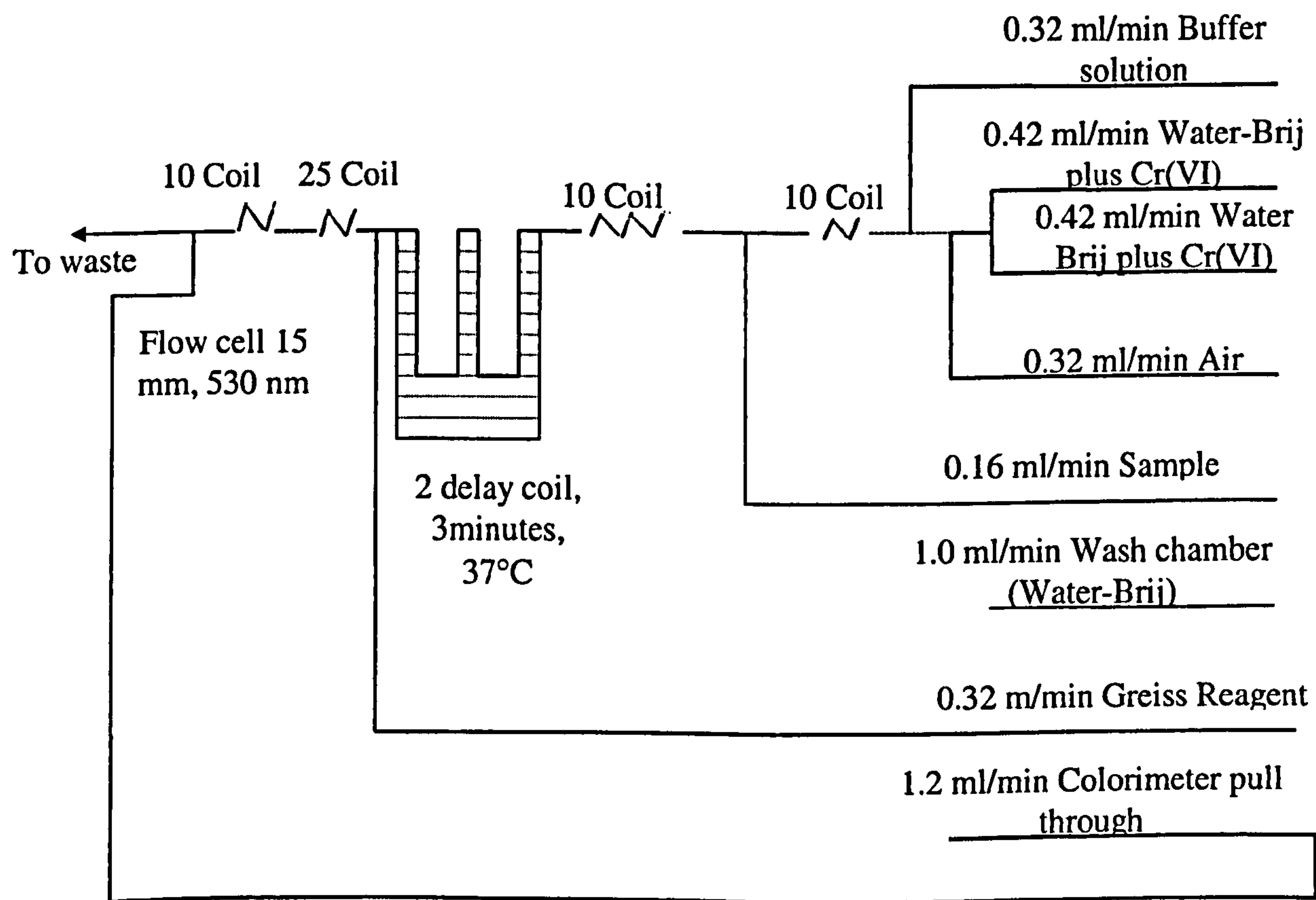


Figure 2.3: System manifold for the determination of nitrite-N.

## **2.4.6 Determination of Nitrate-N in Soil Extracts with 0.5M Potassium Sulphate**

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

### **2.4.6.1 Reagents**

#### **- Buffer solution**

As described in section 2.4.5.1

#### **- Greiss reagent**

As described in section 2.4.5.1

#### **- Reducing reagent**

0.3g of hydrazine sulphate (AR) was weighed into a small beaker. Carefully it was transferred into a 1 litre volumetric flask containing approximately 900ml degassed deionized water. It was then made up to the mark 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. 1ml of 15% Brij-35 solution was added and the solution was mixed gently. If the hydrazine sulphate is shaken in a flask, which is partly filled with deionized water, it will react with the oxygen content of the flask and decreases its reducing power.

#### **- Catalyst solution**

1ml of 2.47 % copper sulphate solution, 10ml of 100mg/l of chromate solution and 1ml 15% Brij-35 solution were added to 1 litre of degassed water and mixed gently.



This solution contains 1mg/l Cr(VI) for nitrate nitrogen analysis of soil extracts from chromium contaminated sites.

**- Nitrate nitrogen stock solution (1000mg/l)**

Sodium nitrate dried at 105°C for 1 hour and cooled in a desiccator. 6.068g of dried sodium nitrate was dissolved in deionized water and the volume was made up to 1 litre. The solution was stored at 2°C.

**- Wash chamber solution**

1ml of 15% Brij-35 was added to 1 litre deionized water and mixed gently.

**- Working nitrate nitrogen standard solutions (0, 1, and 5mg/l)**

Working nitrate nitrogen standard solutions 0, 1 and 5mg/l were prepared using stock solution by dilution it in 0.5M potassium sulphate solution.

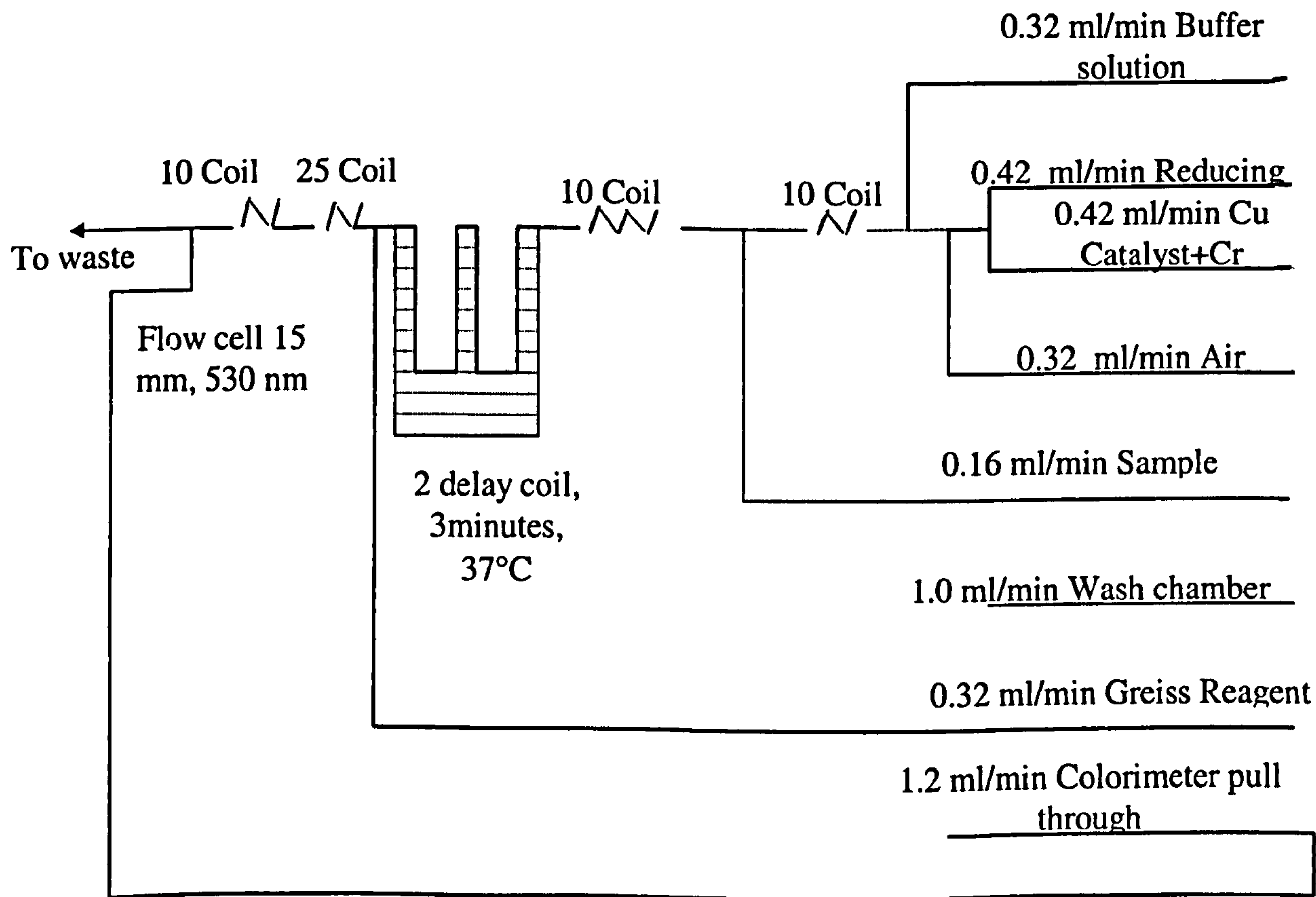


Figure 2.4: System manifold for determination of nitrate-N.

### **2.4.6.2 Procedure**

The Technicon Auto Analyzer II was used for the determination of nitrate nitrogen in 0.5M potassium sulphate. 1mg/l Cr(VI) was added to the copper catalyst solution for nitrate analysis in chromium contaminated sites.

The schematic diagram Figure 2.4 shows the flow system of nitrate nitrogen. The samples were run at the rate of 40 samples per hour and the colour was measured at 530nm. Solutions having concentrations above 5mg/l, were diluted using a dilution step before colour formation.

### **2.4.7 Determination of Available Soil Phosphate in 0.5M Sodium Bicarbonate Extracts with Ascorbic Acid Method**

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

In the ascorbic acid method colour development will occur under acid condition. In the analysis of sodium bicarbonate extract, bicarbonate reacts with acid and produces carbon dioxide gas, which disturbs the system flow. Therefore the samples must be neutralised and all carbon dioxide gas removed, before it is introduced to the colour formation system. The two important factors in the acidification stage are concentration of acid and temperature. The system contains a neutralisation step before colour formation. 2M sulphuric acid solution was suggested by Sarirullah (1989) for the neutralisation at 70°C as this acid was already used in the acid molybdate reagent, therefore, should not interfere with the colour development system.

### **2.4.7.1 Reagents**

#### **- Acid ammonium molybdate**

60ml concentrated sulphuric (AR) acid was added carefully to 800ml deionized water in the fume cupboard and cooled. 5.2g ammonium molybdate (AR) was dissolved in the dilute acid solution. 0.1g antimony potassium tartrate (AR) was dissolved in 100ml of deionized water in a beaker. This solution was then added to the dilute acid ammonium molybdate solution by stirring with a glass rod to avoid precipitation. The volume was made to 1 litre with deionized water. The solution was stored in a dark glass bottle. The solution was then degassed.

#### **- Ascorbic acid solution**

0.75g ascorbic acid (AR) was dissolved in 100ml degassed deionized water. This reagent is unstable and must be prepared on the day of use.

#### **- Wetting agent solution**

For 0-1mg phosphate-P per litre range:

2ml of Aerosol 22 was diluted to 1 litre with degassed deionized water.

For 0-5mg phosphate-P per litre range:

1ml of Aerosol 22 was added to 1 litre with degassed deionized water.

#### **- Neutralising solution (2M sulphuric acid)**

Working in fume cupboard 110ml of concentrated sulphuric acid was added to about 700ml deionized water. It was made up to 1 litre with deionized water after cooling.

#### **- Wash solution**

0.5M sodium bicarbonate pH 8.5 was used as wash solution.



**- Phosphate standard stock solution (1000mg/l)**

Potassium dihydrogen phosphate was dried in an oven at 105°C for one hour. 4.3937g dried potassium dehydrogen phosphate was dissolved in deionized water in a beaker. The content of the beaker were transferred quantitatively to a 1 litre volumetric flask and diluted to the mark with deionized water.

**- Working standard solution (0 and 5mg/l P)**

Stock solution was diluted with 0.5M sodium bicarbonate pH: 8.5 in volumetric flasks to make 0 and 5mg/l standard solutions.

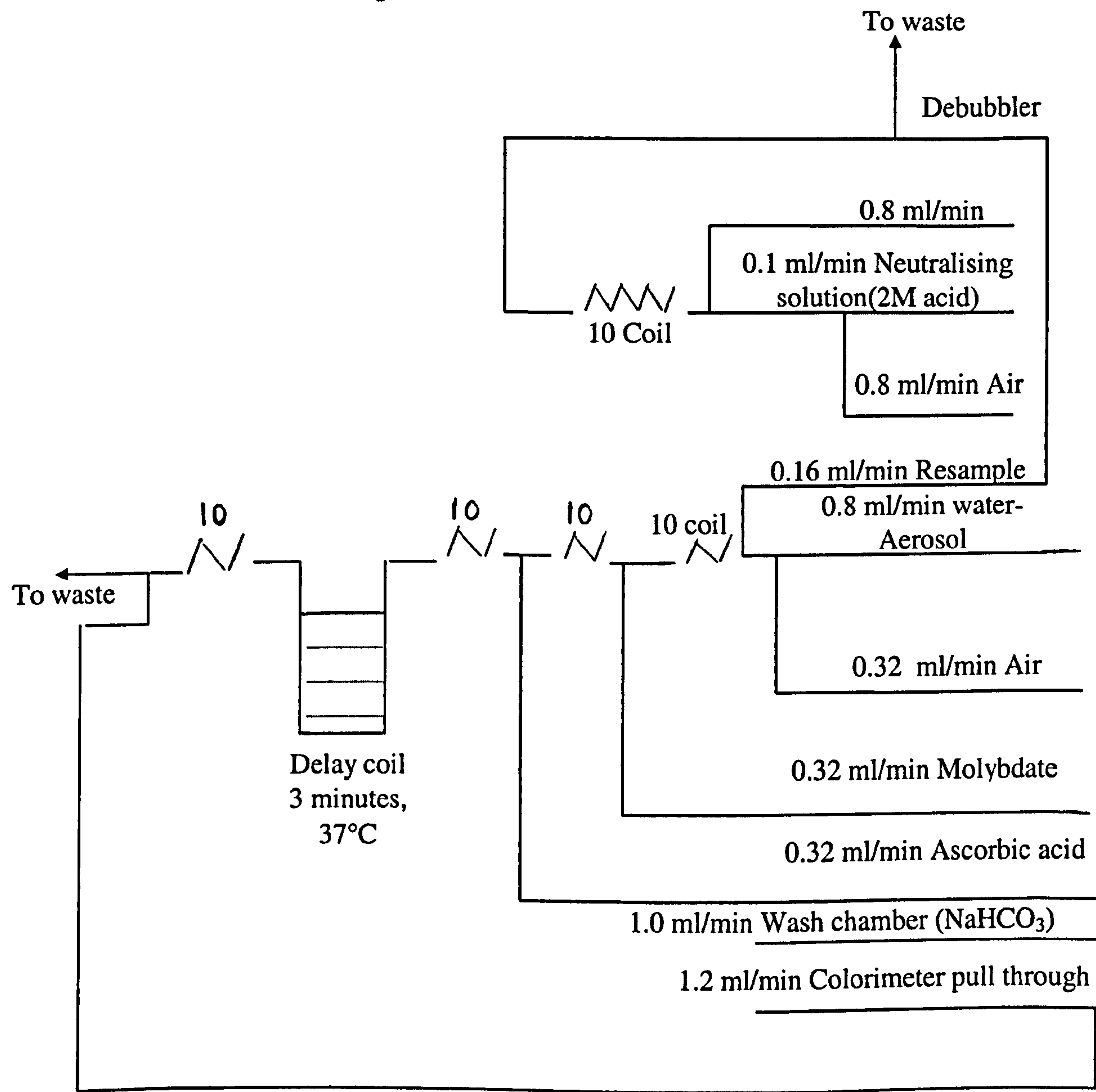


Figure 2.5: System manifold for determination of phosphate-P in bicarbonate extract.



### **2.4.7.2 Procedure**

The system manifold Figure 2.5 used for determination of phosphorus in sodium bicarbonate extracts. The samples were run in Auto Analyser II at 40 samples per hour with a neutralisation-degassing step before the main manifold. The colour formed at 37°C was determined at 880nm. The calibration graph for phosphate-P is linear between 0-5mg/l.

### **2.4.8 Determination of Total Phosphorus in Plant and Soil Perchloric Digest Solutions with Ascorbic Acid Method**

Phosphate was measured using the Technicon Auto Analyzer II. The method is based on the formation of phospho-molybdate complex, which is reduced using ascorbic acid to give a blue colour, which may be measured at 660 or 880nm. In order to speed up the formation of the complex, a small amount of antimony was added. The intensity of blue colour is proportional to the phosphorus concentration in the original solution.

#### **2.4.8.1 Reagents**

##### **- Acid ammonium Molybdate**

As described in section 2.4.7.1

##### **- Ascorbic acid solution**

As described in section 2.4.7.1

##### **- Wetting agent solution**

As described in section 2.4.7.1

**- Phosphate standard stock solution (1000 mg/l)**

As described in section 2.4.7.1

**- Phosphorus working standard solutions (0 and 20 mg/l)**

Phosphorus working standard solutions 0 and 20 mg/l were prepared in the 5% perchloric acid.

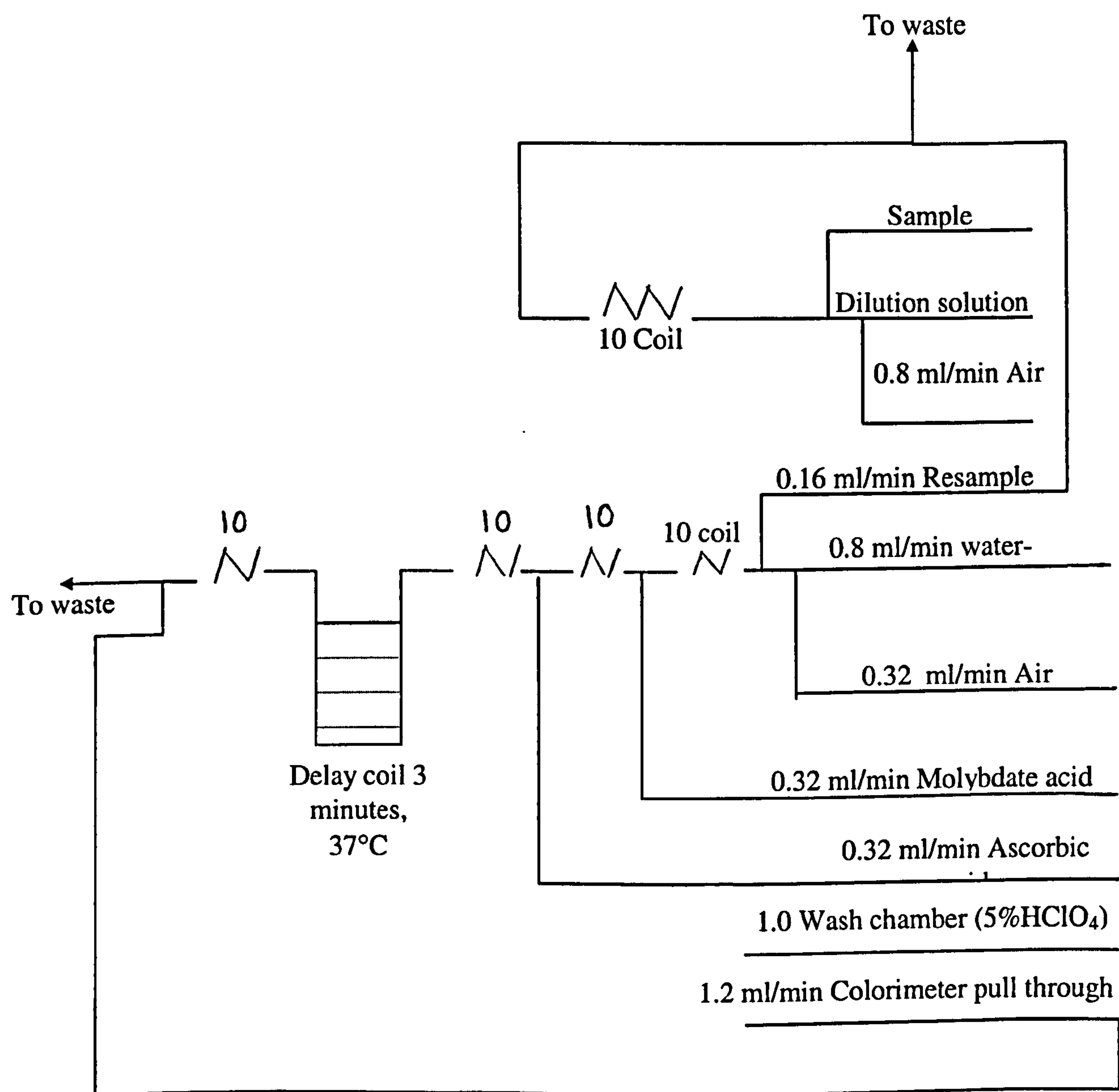


Figure 2.6: System manifold for determination of phosphate-P in digest solution by ascorbic acid method.

#### **- Dilution solution**

Water was used as diluter solution in perchloric acid digest solution analysis with ascorbic acid.

#### **- Wash chamber solution**

5% perchloric acid was used as wash chamber solution.

### **2.4.8.2 Procedure**

The digest solutions were analysed using the Technicon Auto Analyzer II manifold showed in Figure 2.6, along with standard solutions, blanks and zeros. The samples were run at the rate of 40 per hour and the colour was developed in the water bath at 37°C. The technicon Auto Analyzer main manifold can measure phosphate in a linear calibration between 0-5 mg/l phosphate-p. A dilution system was necessary to decrease the concentration of solutions to adjust the linear calibration in the Technicon Auto Analyzer.

The colour intensity was measured at 880nm.

### **2.4.9 Determination of Potassium in M Ammonium Nitrate Extracts**

The concentration of potassium in the soil extracts was determined by flame photometer Corning-Model: 410.

#### **2.4.9.1 Reagents**

##### **- Potassium stock standard solution (1000mg/l)**

Potassium stock solution was supplied by BDH Spectrosol.

### **- Potassium working standard solution**

Solutions containing 0, 10, 20, 30, 40 and 50mg/l potassium were prepared in a matrix of M ammonium nitrate.

### **2.4.9.2 Procedure**

The flame photometer was set according to the manufacturer's instructions, to measure potassium emission.

The potassium working standard solutions containing 0 and 50mg/l were aspirated and the controls adjusted to give steady zero and maximum readings. The intermediate working standard solutions were nebulised and a graph relating readings to concentration of standard solutions was constructed.

### **2.4.9.3 Testing of Extracts**

The flame photometer was adjusted until steady zero and maximum reading are obtained with potassium working standard solutions containing 0 and 50mg/l of potassium, as described above. The extracts were nebulised and the meter readings were recorded.

### **2.4.9.4 Calculation**

From the constructed graph the concentration of potassium in solution was obtained. The concentration of potassium in soil calculated as follow:

$$A = \frac{B \times 50}{W}$$

Where:

- A: Concentration of potassium in soil in *mg/k*
- B: Concentration of potassium in soil extract in *mg/l*
- W: The weight of used soil in *g*



50: The volume of soil extract in *ml*

## **2.4.10 Determination of Total Potassium in Soil and Plant**

The concentration of total potassium was determined in digest solutions of soil or plant, which were digested with concentrated perchloric acid. The determination was carried out using the flame photometer Corning-Model: 410.

### **2.4.10.1 Reagents**

#### **- Potassium stock solution (1000mg/l)**

The 1000mg/l potassium stock solution was supplied by BDH Spectrosol.

#### **- Potassium working standard solutions**

Working standard solutions 0, 10, 20, 30, 40 and 50mg/l were prepared in 5% perchloric acid.

### **2.4.10.2 Procedure**

The procedure, test of digest solution and calculation were done as described in sections 2.4.9.2, 2.4.9.3 and 2.4.9.4.

## **2.4.11 Determination of Cr(VI), NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N in Soil Water Extracts with Ion Chromatography**

Chromate, nitrite and nitrate were determined in soil water extracts by Ion Chromatography (Model: Dionex DX 500 Ion Chromatography System).

The system contained of:

1 - GP40 Gradient Pump.

2 - LC10 Chromatography Module (AG11 Guard column and AS11 Separator column and ASRS Anion Self Regenerating Suppressor).

3 - ED40 Electrochemical Detector in conductivity mode.

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

### **2.4.11.1 Reagents**

#### **- Chromate ion stock solution [1000mg/l Cr(VI)]**

Potassium chromate was dried at 102°C for 1 hour. 3.7349g of dried salt was dissolved in deionized water and made up to 1 litre with deionized water.

#### **- Nitrite nitrogen stock solution (1000mg/l)**

As described in section 2.4.5.1.

#### **- Nitrate nitrogen stock solution (1000mg/l)**

As described in section 2.4.6.1.

#### **- Working standard solution (0 and standard solution 1mg/l Cr(VI) plus 1mg/l NO<sub>2</sub><sup>-</sup>-N plus 1mg/l NO<sub>3</sub><sup>-</sup>-N)**

Deionized water was used as 0 standard solution. Standard solution containing 1mg/l Cr(VI) and 1mg/l NO<sub>2</sub><sup>-</sup>-N plus 1mg/l NO<sub>3</sub><sup>-</sup>-N was also prepared in 100ml volumetric flasks with deionized water.

### **2.4.11.2 Procedure**

The determination was carried out with Ion Chromatography system Dionex DX 500. Duplicate standard solutions were injected for initial calibration of the

instrument. Then the soil water extracts, blanks and standards were run through the IC. Chromate, nitrite and nitrate were determined in a same run.

**2.4.11.3 Method of Separation**

The three anions in water extract were separated by gradient separation. The gradient separation used in this determination is given in the Table 2.2.

Time (Minutes)	Eluent A (Water) %	Eluent B (200 mM NaOH) %
0	97.5	2.5
1.0	97.5	2.5
10.0	85.0	15.0
10.10	97.5	2.5
15.00	97.5	2.5

Table 2.2: Gradient separation used for determination of chromate, nitrite and nitrate by Ion Chromatography.

**2.4.11.4 Method Parameters**

The parameters used in this determination are as follow:

Eluent flow rate = 2.0ml/min

Injection loop = 25.0µl

Detector mode: Conductivity

SRS Current: 100mA

The retention and standard calibration details are given in Table 2.3.

Component	Retention Time (minutes)	Standard Concentration (mg/l)
Nitrite	1.87	1.00
Nitrate	3.11	1.00
Chromate	7.45	1.00

Table 2.3: Retention time and standard concentrations used in determination of chromate, nitrite and nitrate by Ion Chromatography.



## CHAPTER THREE

### ANALYTICAL METHOD DEVELOPMENT AND EVALUATION

This chapter deals with problems with nitrite-N and nitrate-N analysis in chromium contaminated soil extracts, phosphate analysis in digest solutions and finally evaluation of digestion methods for phosphate-P determination in vegetation samples.

#### **3.1 A Problem in the Determination of Nitrite-N and Nitrate-N in Chromium Contaminated Soil Extracted with 0.5M Potassium Sulphate**

##### **3.1.1 Introduction**

Determinations of nitrite-N and nitrate-N in 0.5M potassium sulphate soil extracts were carried out by Technicon Auto Analyzer II. During these experiments there was a problem with the chart recorder peaks. Some soil extracts especially those from highly contaminated sites, which were yellow coloured, had very badly shaped peaks with bad carry over effects. It was assumed that chromium may have affected the peaks. This effect might be because of the background colour of extracts, or interference by chromium itself in the determination of nitrite-N and nitrate-N. In the automated system nitrate-N is quantitatively reduced to nitrite-N followed by determination of the nitrite using the Griess reagent. The method, therefore, measures nitrate plus nitrite. The nitrite nitrogen can be measured separately on the same manifold by omitting the reduction reagents. Since the problem with the peaks happened both in nitrite-N and nitrate-N analyses and there is no reduction part in nitrite-N analysis, the problem might not be in the reduction part of the analysis with the Griess reagent.



Therefore in order to find out the cause of the problem, it was decided to measure the chromium level of the extracts and evaluate the degree of background colour or interference with the chemistry of the analysis.

### **3.1.2 Materials and Methods**

Nineteen soil samples were collected from different depths (0-15cm, 15-30cm, 30-50cm) of six sampling points. Sample number 19 was collected from more than 50cm depth of sampling point 3. Sampling points are described in section 5.2.1.5 and Figure 4.11.

#### **3.1.2.1 Preliminary Measurements**

Soils were extracted with 0.5M potassium sulphate as it is described in section 2.2.1. Nitrite-N, nitrate-N, chromium level in soil extracts and preliminary effect of different levels of chromium on nitrite nitrogen and nitrate nitrogen systems were carried out.

#### **Analysis of nitrite-N and nitrate-N in soil extracts**

These analysis were done as described in section 2.4.5 and 2.4.6.

#### **Reagents**

Conventional methods have been used in this section. Due to certain problems some changes were made to the method which is explained in this section. The final version of the reagents has been explained in section 2.4.5 and 2.4.6. The following unmodified version of reagents was used here:

##### **- Catalyst solution**

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

## **- Water Brij**

1ml of Brij-35% was added to 1 litre of deionized water and mixed well.

## **Analysis of Chromium**

The soil extract solutions were analyzed by Atomic Absorption Spectrophotometer as described in section 2.4.1, to find out the concentration of chromium which can be extracted along with nitrite-N and nitrate-N with 0.5M potassium sulphate.

## **Preliminary study of the effect of chromium on nitrite-N and nitrate-N analysis**

Three sets of standard solutions of chromium containing 0, 10 and 100mg/l Cr(VI) prepared in the 0.5M potassium sulphate solution were made as follows:

- a - Without nitrite and nitrate.
- b - With 1mg/l nitrite each.
- c - With 1mg/l nitrate each.

Two soil extracts showing severe interference problems were diluted in different degrees and run for comparison.

The solutions run for nitrite-N were as follows:

- 0mg/l NO<sub>2</sub>-N plus 0mg/l Cr(VI).
- 0mg/l NO<sub>2</sub>-N plus 10mg/l Cr(VI).
- 0mg/l NO<sub>2</sub>-N plus 100mg/l Cr(VI).
- 1mg/l NO<sub>2</sub>-N plus 0mg/l Cr(VI).
- 1mg/l NO<sub>2</sub>-N plus 10mg/l Cr(VI).
- 1mg/l NO<sub>2</sub>-N plus 100mg/l Cr(VI).
- Soil sample extract No: 9 with 1/5 Dilution.
- Soil sample extract No: 9 with 1/10 Dilution.
- Soil sample extract No: 19 with 1/5 Dilution.
- Soil sample extract No: 19 with 1/10 Dilution.
- Soil sample extract No: 19 with 1/25 Dilution.

The solutions run for nitrate were as follows:

- 0mg/l NO<sub>3</sub>-N plus 0mg/l Cr(VI).
- 0mg/l NO<sub>3</sub>-N plus 10mg/l Cr(VI).
- 0mg/l NO<sub>3</sub>-N plus 100mg/l Cr(VI).
- 1mg/l NO<sub>3</sub>-N plus 0mg/l Cr(VI).
- 1mg/l NO<sub>3</sub>-N plus 10mg/l Cr(VI).
- 1mg/l NO<sub>3</sub>-N plus 100mg/l Cr(VI).
- Soil sample extract No: 9 with 1/5 Dilution.
- Soil sample extract No: 9 with 1/10 Dilution.
- Soil sample extract No: 19 with 1/5 Dilution.
- Soil sample extract No: 19 with 1/10 Dilution.
- Soil sample extract No: 19 with 1/25 Dilution.

### **3.1.2.2 The Effect of Background Colour on the Nitrite-N System**

In the nitrite-N manifold the Griess reagent was replaced with 10% hydrochloric acid in order to see the effect of only background colour of chromium on the peaks in this system.

The solutions, which were run, were in this order:

- 0mg/l Cr(VI) plus 0mg/l NO<sub>2</sub>-N.
- 10mg/l Cr(VI) plus 0mg/l NO<sub>2</sub>-N.
- 100mg/l Cr(VI) plus 0mg/l NO<sub>2</sub>-N.
- Soil sample extract No: 9 with 1/5 Dilution.
- Soil sample extract No: 9 with 1/10 Dilution.
- Soil sample extract No: 19 with 1/5 Dilution.
- Soil sample extract No: 19 with 1/10 Dilution.
- Soil sample extract No: 19 with 1/25 Dilution.



### **3.1.2.3 The Effect of Chromium on Chemistry (Colour Development) in Nitrite-N Analysis System**

Two sets of the working standard solutions containing 0, 0.2, 0.4, 0.6, 0.8, 1.2, 4, 6, 8 and 10mg/l Cr(VI) were prepared in 0.5M potassium sulphate. Nitrite was added to one set to make the concentration of 1mg/l nitrite-N in the solutions. No nitrite was added to another set. These sets were analyzed with the Technicon for nitrite-N.

### **3.1.2.4 The Effect of Background Colour on Nitrate-N system**

The same experiment was carried out as described in section 3.1.2.2, but nitrate standard and nitrate-N manifold was used instead of nitrite standard and nitrite-N manifold.

### **3.1.2.5 The Effect of Chromium on Chemistry (Colour Development) in Nitrate-N Analysis System**

The same experiment was carried out as described in section 3.1.2.2, but nitrate standard and nitrate-N manifold was used instead of nitrite standard and nitrite-N manifold.

### **3.1.2.6 The Effect of Cr(VI) on Nitrite-N and Nitrate-N Systems Containing Cr(VI) in Reagent**

The copper catalyst and water-Brij containing 1mg/l and 0.5mg/l chromium respectively, were used as described in section 2.4.5 and 2.4.6.

The two experiments were done as described in section 2.4.5 and 2.4.6.

Solutions of section 3.1.2.3 and 3.1.2.5, were run with the chromium added to the reagents.



### 3.1.3 Results and Discussion

During the determination of nitrite-N and nitrate-N there was a problem with the chart recorder peaks. Some soil extracts had very badly shaped peaks with bad carry over effects.

After analysis of chromium in the extracts, it was found that the chromium content of the soil extracts, which are shown in Table 3.1, were less than 1mg/l, except for samples 9 and 19 which had a bright yellow colour and high chromium content.

Soil Number	Cr.in K <sub>2</sub> SO <sub>4</sub> Extract (mg/l)	Soil Number	Cr.in K <sub>2</sub> SO <sub>4</sub> Extract (mg/l)
1	0.1	11	0.48
2	0.16	12	0.40
3	0.88	13	0.16
4	0.59	14	0.14
5	0.39	15	0.13
6	0.31	16	0.10
7	0.16	17	0.10
8	0.60	18	0.17
9	93	19	495
10	0.57		

Table 3.1 Chromium content of 0.5M potassium sulphate soil extracts.

The analysis of standard solutions spiked with 0, 10 and 100mg/l Cr(VI) and dilution of the two soil extracts showed the same problems with badly shaped and carry over effects of chromium in this system (Tables 3.2 and 3.3). The problem was worse with the higher concentration of chromium.

mg/l Chromium	0mg/l NO <sub>2</sub> <sup>-</sup> -N	0mg/l NO <sub>3</sub> <sup>-</sup> -N	1mg/l NO <sub>2</sub> <sup>-</sup> -N	1mg/l NO <sub>3</sub> <sup>-</sup> -N
0	O.K	O.K	O.K	O.K
10	O.K	O.K	Affected	Affected
100	O.K	O.K	Affected	Affected

Table 3.2: The peaks of nitrite-N and nitrate-N of the standard solutions.

Soil Extract (dilution)	NO <sub>2</sub> <sup>-</sup> -N Analysis	NO <sub>3</sub> <sup>-</sup> -N Analysis
Sample 9 (1/5)	Affected	Affected
Sample 9 (1/10)	Affected	Affected
Sample 19 (1/5)	Affected	Affected
Sample 19 (1/10)	Affected	Affected
Sample 19 (1/15)	Affected	Affected

Table 3.3: The peaks of nitrite-N and nitrate-N of the diluted soil samples.

Replacing the Griess reagent with 10% hydrochloric acid in the colour development reaction, showed that there was no interference from background colour of chromium in the solutions containing up to 10mg/l chromium.

Figure 3.1 shows the peak heights from the standard solutions containing chromium (0-10mg/l) with and without 1mg/l nitrite-N. There was a slight increase in the peaks of the chromium standards without nitrite-N with increase with chromium concentration. The figure also shows that the peaks for the standard chromium solution with 1mg/l nitrite also increased with increase in the concentration of chromium. The plotted graph showed a steep response to chromium concentration between 0-0.2mg/l with less response to addition of higher levels of chromium. It means the system is more sensitive to the first addition of chromium. This indicated that the chromium had an effect on the nitrite-N system and caused overestimation of nitrite-N, which in solutions with low concentrations of nitrite can cause big errors. The peak heights of the 1mg/l nitrite-N solution showed a 10% increase on increasing the chromium concentrations to 1mg/l, and 19% increase at 10mg/l chromium.

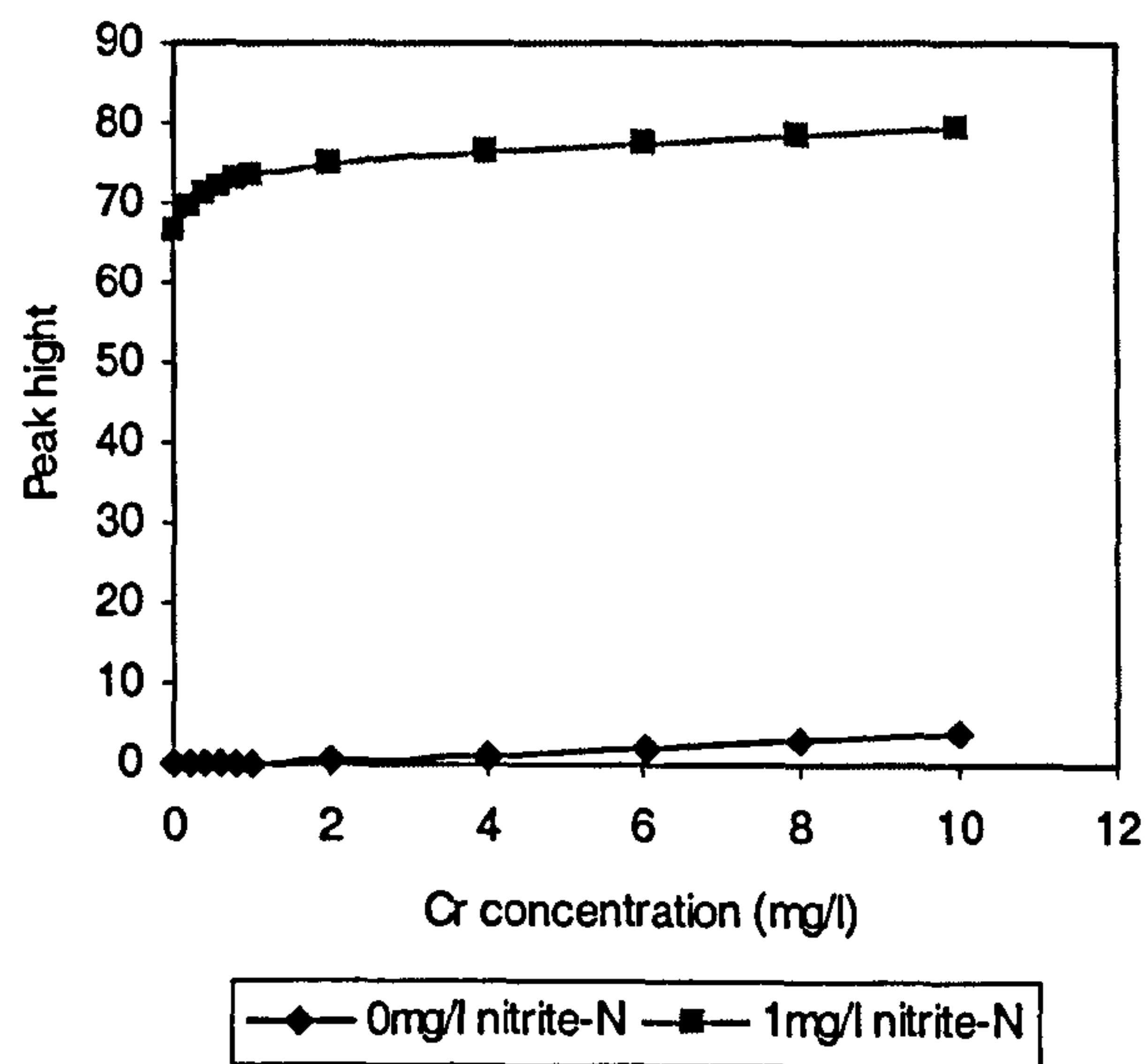


Figure 3.1: Changes in peak height of 0 and 1mg/l nitrite-N at varying chromium concentration.

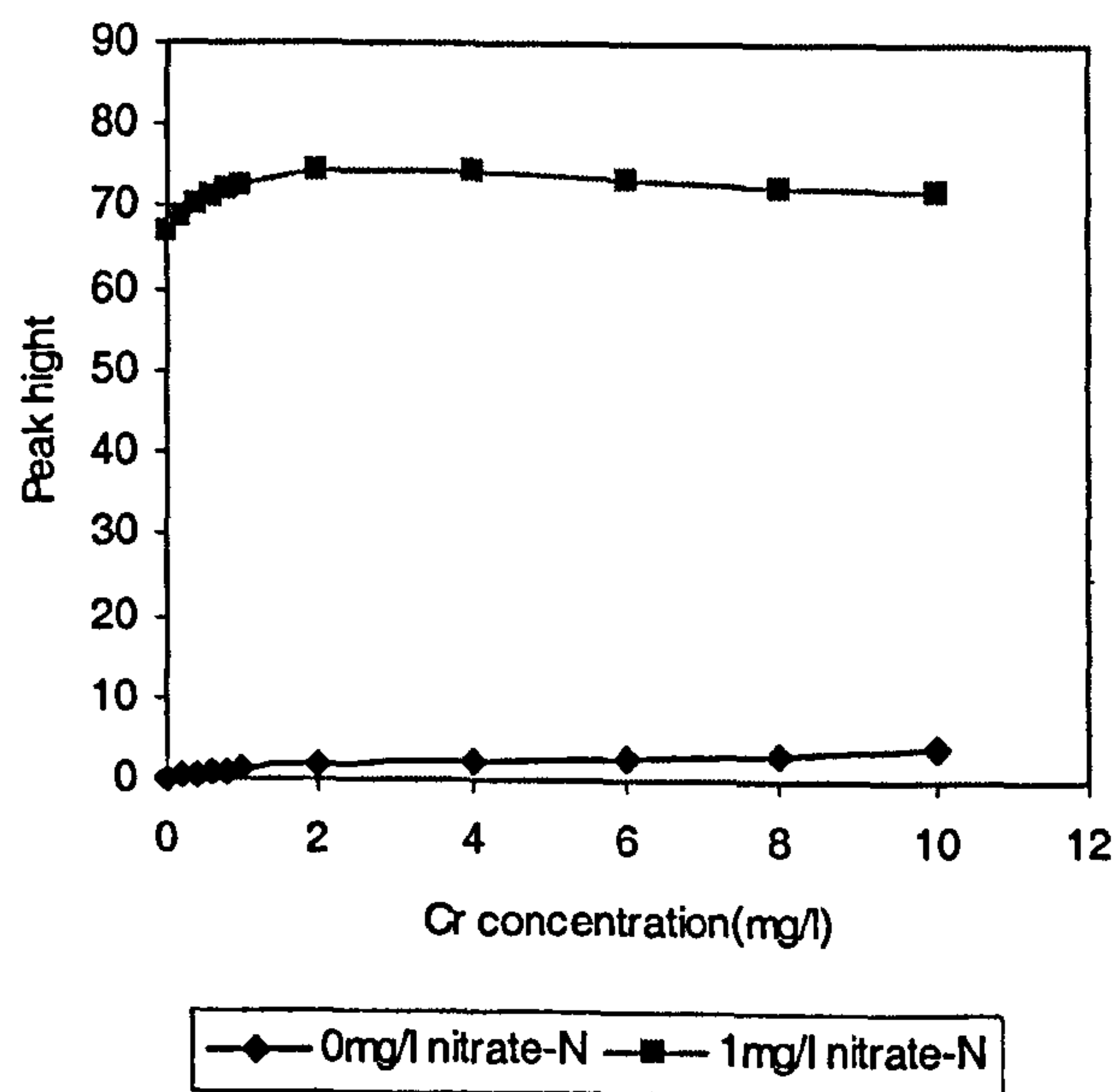


Figure 3.2: Changes in peak height of 0 and 1mg/l nitrate-N at varying chromium concentration.



Figure 3.2 shows the peak heights from standard solutions containing chromium (0-10mg/l) with and without 1mg/l nitrate-N. This graph shows that the chromium had the same effect on the nitrate-N system as it had on the nitrite-N system, except it was followed by a reduction of peaks and a negative effect at higher chromium concentrations. The peak heights of 1mg/l nitrate-N increased by 8% on increasing the chromium concentrations to 1mg/l.

Since the small differences at low concentration of chromium cause large errors in the nitrite-N and nitrate-N analysis, it was decided to maintain a constant low level of chromium in the systems while analysis of the extract solutions was in progress.

For this purpose chromium was added to water-Brij reagent at 0.5 mg/l, which is equivalent to approximately 2.5mg/l chromium in the sample solution. This made possible the analysis of nitrite-N with negligible errors with small differences in sample chromium concentrations.

Figure 3.3 shows the peak heights, for chromium standard solutions with and without 1mg/l nitrite in the new system. When water Brij reagent containing 0.5 mg/l Cr(VI) was used instead of the conventional water Brij reagent in this analysis, the obtained results showed that the difference between the peaks of solutions with and without chromium were reduced. The effect of 1mg/l nitrite-N peaks decreased from about 10% in the previous system to 1% in the new system up to 1mg/l chromium concentration. This effect was 10% at up to 10mg/l chromium in new system compared with 19% in the previous system. Adding the chromium to the reagent reduced the interference, by low additions of chromium solution, allowing chromium contaminated soil extract solutions to be run for nitrite-N by this new system.

Since the concentration of chromium in the extract of soil samples with high chromium content is high (for example soil samples 9 and 19), their nitrite-N can not be measured by this system. The amount of nitrite-N in these soil extracts can be measured by Ion Chromatography.



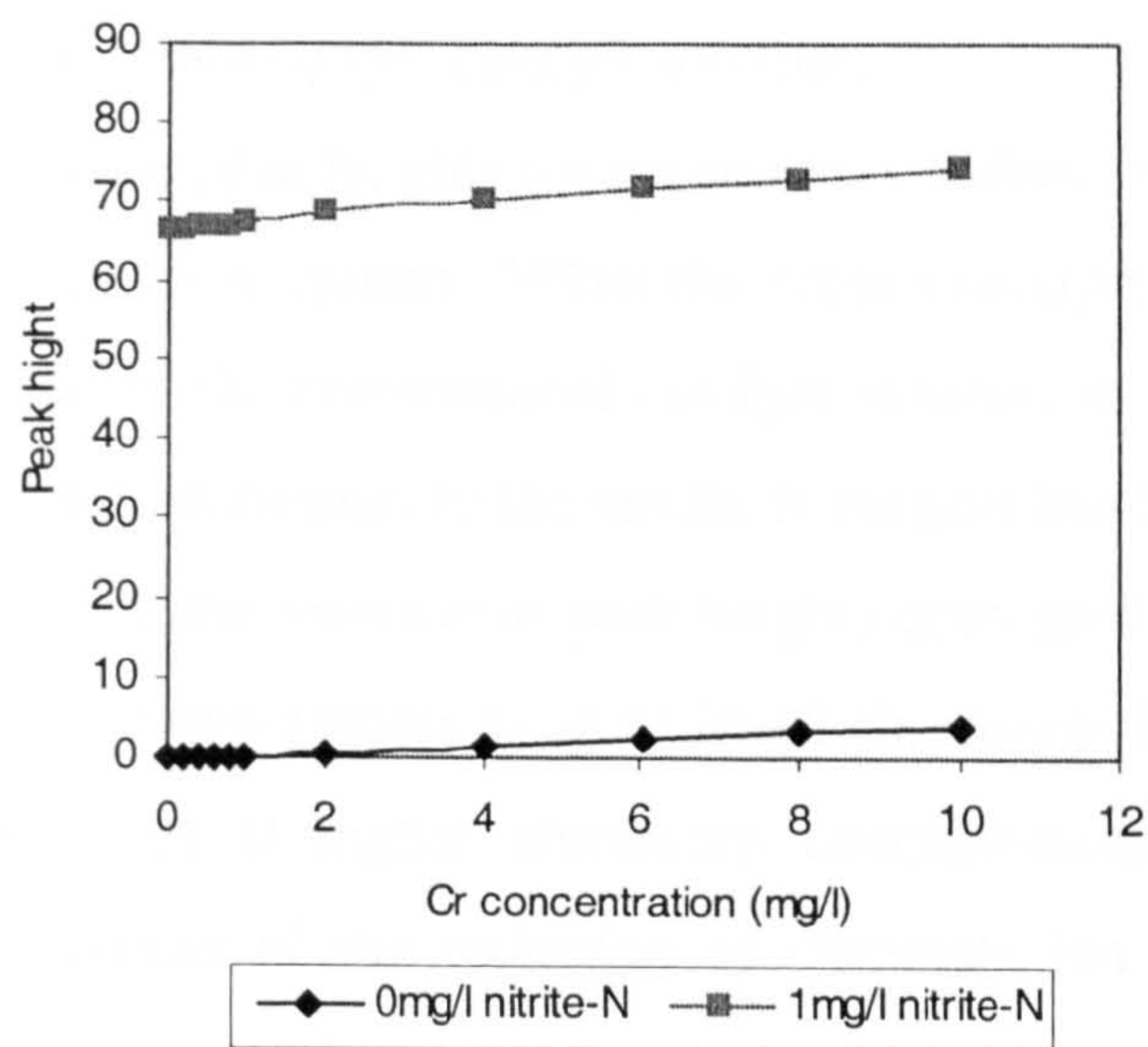


Figure 3.3: Changes in peak height of 0 and 1mg/l nitrite-N at varying chromium concentration in new system.

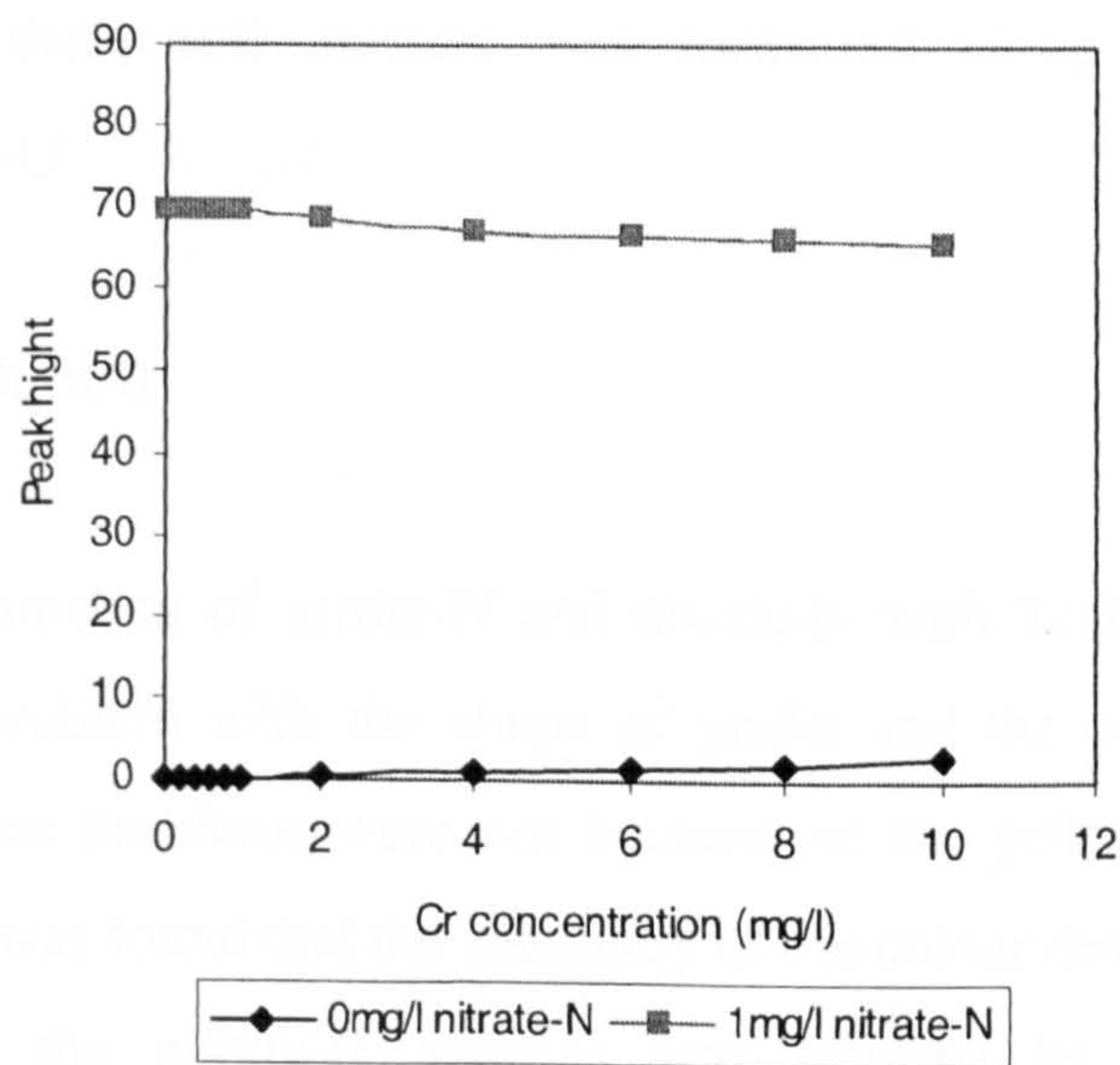


Figure 3.4: Changes in peak height of 0 and 1mg/l nitrate-N at varying chromium concentration in new system.

In the nitrate-N manifold for the same reason as for the nitrite-N system 1mg/l chromium was added to copper catalyst solution.

Figure 3.4 shows the peak heights for chromium standard solutions with and without 1mg/l nitrate in the new system. When the copper catalyst containing 1mg/l Cr(VI) was used instead of the conventional catalyst solution in this analysis, the results showed that adding chromium to the nitrate-N reagent had the same effect as for the nitrite-N system and the increase in peak heights decreased from 8% in the previous system to 0% in the new system at up to 1mg/l chromium, however, it was followed by a negative effect at higher chromium concentrations. The negative effect probably was because of the reduction of chromate ion caused by the reducing reagent in the system.

Adding the chromium to the reagent in nitrate-N system also reduced the interference in the system caused by low levels of chromium, allowing chromium contaminated soil extracts also be analyzed with the new nitrate-N system.

As was mentioned for the nitrite-N analysis, the nitrate-N also could not be measured in solutions with high content of chromium by this system. The amount of nitrate-N in these soil extracts was measured along with nitrite-N by Ion Chromatography.

### **3.1.4 Conclusion**

During determination of nitrite-N and nitrate-N with Technicon Auto Analyzer II there was a problem with the shape of peaks and the carry over. Experiments showed that the problems were not because of the yellow background colour of chromium. It was found that the chemistry of the colour development reaction of the nitrite-N and the nitrate-N systems was affected by chromium and caused overestimation of nitrite-N and nitrate-N. An addition of 0.5mg/l Cr(VI) to the water Brij reagent in the nitrite-N system and 1mg/l Cr(VI) in the copper catalyst solution in the nitrate-N system can reduce the effects of this problem at low concentrations of chromium, allowing analysis of  $\text{NO}_2^-$ -N/ $\text{NO}_3^-$ -N in solutions containing up to 1mg/l chromium.



## **3.2 Problems of Background Colour in Determination of Total Phosphate-P in Plant Digest Solutions**

### **3.2.1 Introduction**

A study was done on the determination of total plant phosphate-P in this department using the conventional Kjeldahl method. The recoveries of P from the certified plant materials were higher than certified values. It was therefore decided to carry out some experiments on total plant phosphorus determination. There are several different digestion methods with varying single or mixtures of acids. The digestion solutions are then analysed for phosphate-P. There are two main colorimetric analysis methods for phosphate-P analysis, the ascorbic acid and the molybdate-metavanadate methods.

In the molybdate-metavanadate method of analysis, phosphate-P makes a yellow colour as a phospho-vanado-molybdate complex and the intensity of this yellow colour is measured as the concentration of phosphate-P at 420nm in the range 0-50mg/l

The plant digests with nitric acid had a yellow colour. During determination of phosphorus in the digest solution with concentrated nitric acid, while the molybdate-metavanadate was used as an analytical method, some problems appeared in peak heights. Two replicates of the same digest solution following standards and blanks did not have the same peak heights. The first peak was lower than the second one. Two replicates of top standards following the yellow digest solution had the same problem, but in this case the peak of the first replicate of the standard was higher than the second one. It was clear that these kind of problems appeared when colourless solutions were followed by a yellow coloured sample solution or yellow coloured sample solutions were followed by colourless solutions. It was considered that these problems were probably because the yellow colour was sticking to inside of the tubes of Auto Analyzer system, but was released into more dilute solution. In order to investigate the systematic errors in phosphorus analysis in the concentrated nitric acid digest solutions, a number of experiments were carried out.

### **3.2.2 Materials and Methods**

Two certified plant materials hay and cabbage along with one grass sample were used in this study. The plants were digested with concentrated nitric acid method as described in section 3.4.3.1. The digest solutions were analyzed with molybdate-metavanadate analytical methods as described in section 3.4.4.2.

#### **3.2.2.1 Finding the Site of the Problem**

Different parts of the sample tubing of the system were replaced with glass or silicon rubber and solutions were run with the replaced tubes to find out the exact place in which the yellow colour had been stuck.

#### **3.2.2.2 Effect of the Acid Concentration**

Metavanadate manifold as is described in section 3.4.4.2, includes:

- Sample tube: 0.32ml/min
- Metavanadate tube: 0.32ml/min
- Nitric acid tube: 1ml/min

Therefore the final volume of flow is 1.64ml/min and the concentration of acid changes at the final coil of the system. In the conventional method 2% nitric acid is used for this system. Experiments were carried out to see the effect of acidity on the background colour due to the digests and the reagents.

##### **- Effect on background colour of molybdate/metavanadate**

The metavanadate reagent has also a yellow colour. Different nitric acid solutions with concentrations of 0, 1, 2, 3, 4, 5, 6, 8 and 10% were made. An experiment was carried out to see the effect of different acid concentration on the background colour of metavanadate reagent.



### **- Effect on the background colour of the digest samples**

In the metavanadate analysis system metavanadate reagent was replaced with deionized water. Sample solutions were then run without the colour formation reagent using above different nitric acid reagent solutions.

### **- Effect on the colour formation of phosphorus in sample**

The sample solutions were run by the system with the colour formation reagent (metavanadate), using the above nitric acid reagents with different acid concentration.

## **3.2.3 Results and Discussion**

It was found that the background colour stuck to the PVC tubing and did not stick to the glass or the silicon rubber tubing.

Silicon rubber tubing was used in the sample line from the automatic sampler to the pump, pump tube, and from the pump to the manifold instead of the PVC tubing and the glass tube was used for connection of the manifold to the colorimeter.

Figure 3.5 shows the background colour of the molybdate-metavanadate reagent decreased at until 2-3% nitric acid but did not change after approximately 3% (0.5M) final concentration of nitric acid.

Figure 3.6 shows the change of background colour of four different replicates of 3 plant samples with acid concentration. In the obtained results the values of peak heights continued to fall as acid concentration was increased at all levels tested, however, the fall was less steep at acid concentrations above approximately 3.5%.

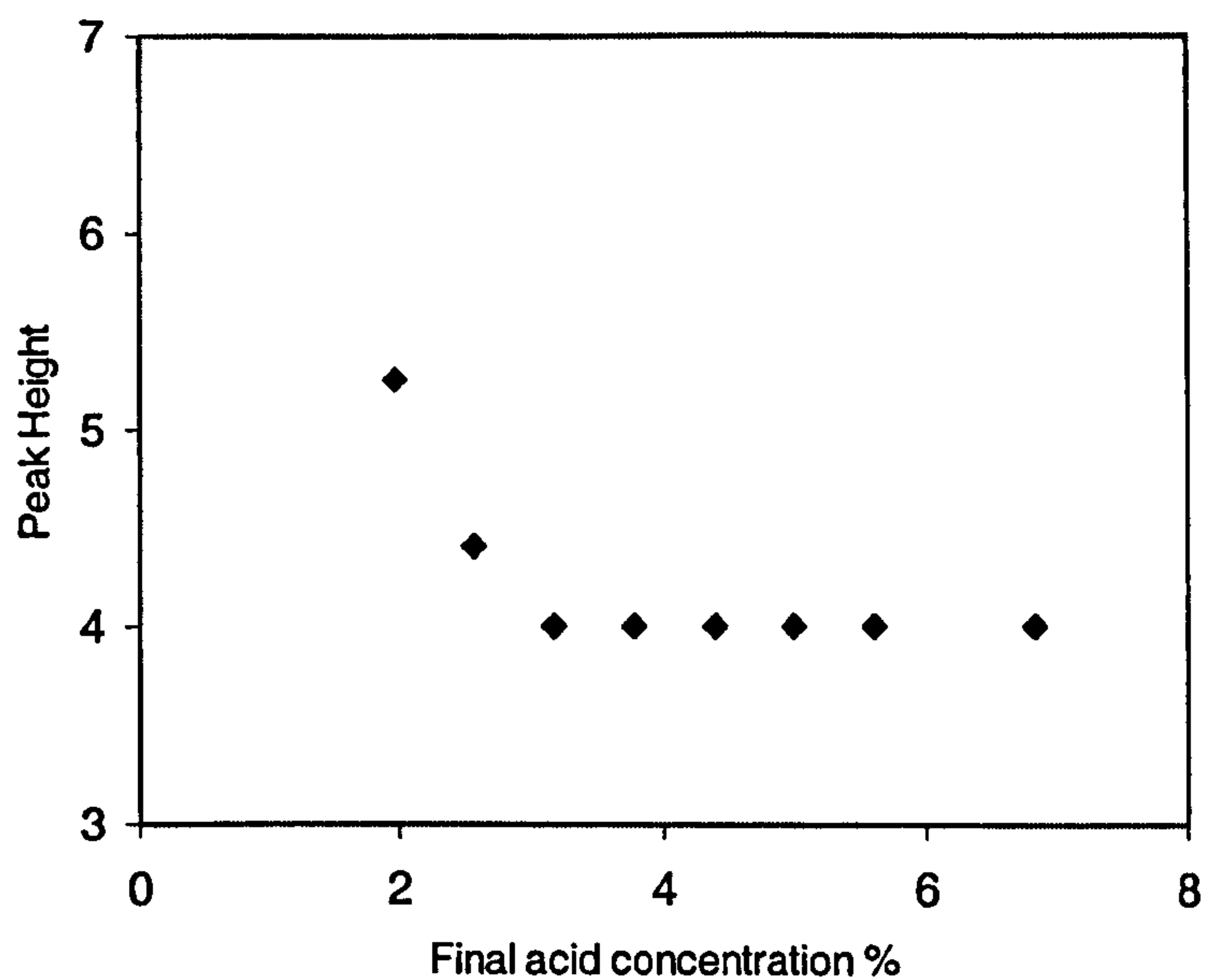


Figure 3.5: The Effect of the final acid concentration on the background colour of the metavanadate reagent.

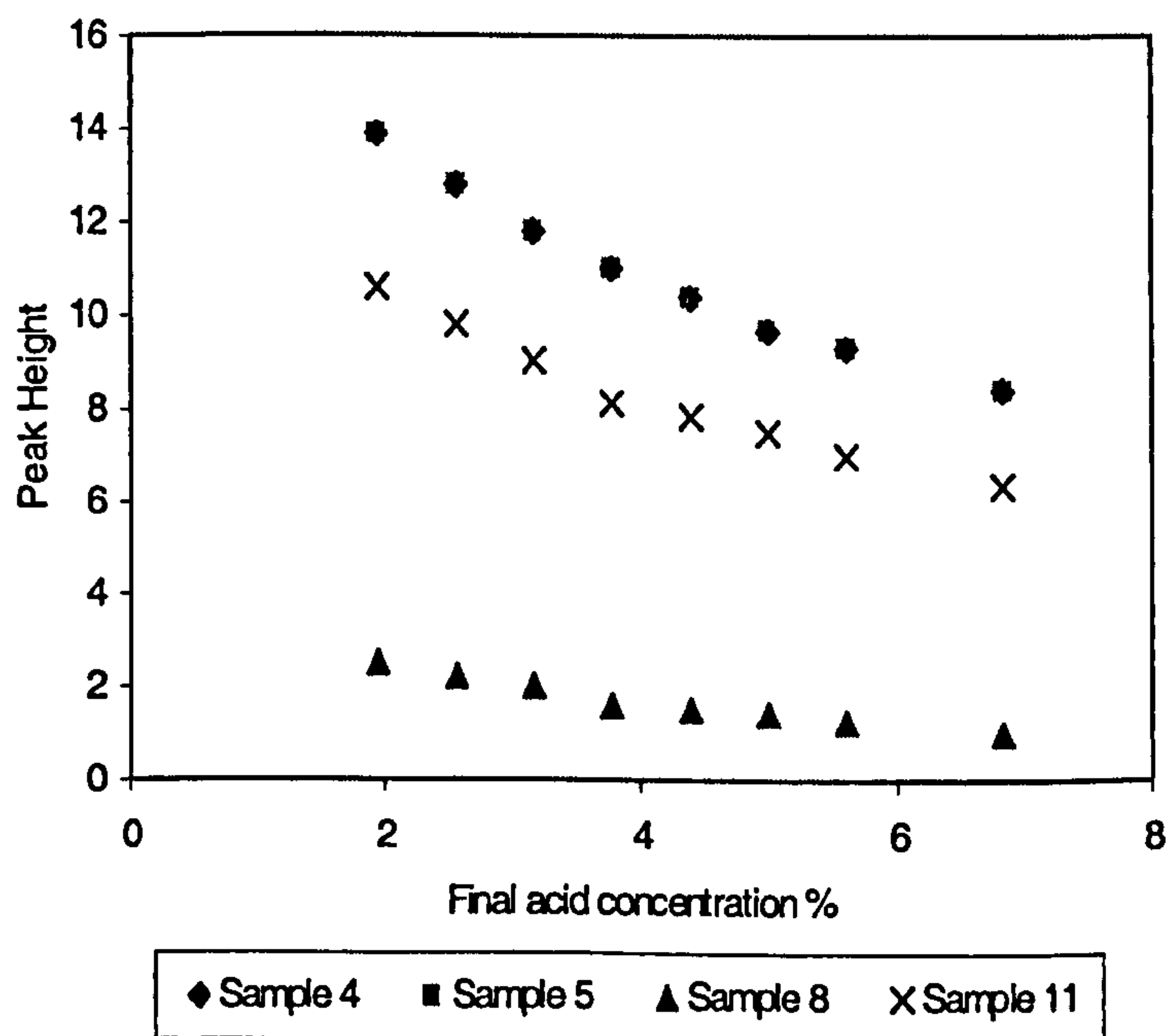


Figure 3.6: The effect of the final acid concentration on the background colour of some samples.

As is shown in Figure 3.7 the yellow colour of phosphate-metavanadate complex plus yellow background colour of samples also decreased with acid concentration, but was less steep at greater than 3.5% nitric acid concentration.

The results obtained for phosphorus determination peak height in plant material show a decrease with acid concentration up to 3.5%. The graph demonstrates steady state above about 3.5% (0.5M) final acid concentration. The experiment showed that many parameters changed the slope of graph at 3.5% final acid concentration. Therefore to avoid errors due to small differences in nitric acid concentration, it was decided to choose 3% nitric acid in the nitric acid reagent tube. This corresponds to 3.7% (0.6M) final nitric acid concentration for analysis of phosphate-P in the molybdate-metavanadate method.

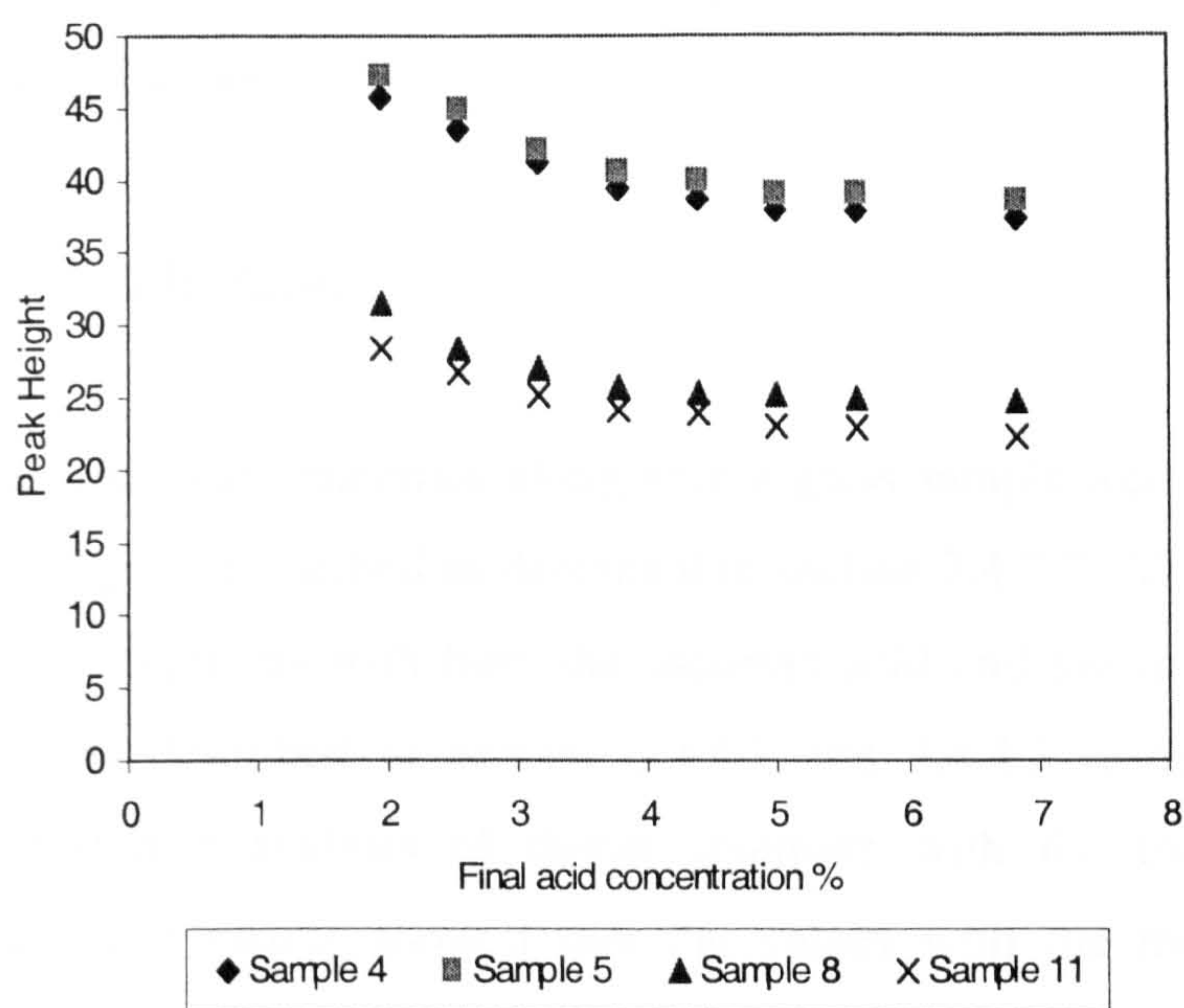


Figure 3.7: The effect of the final acid concentration on the phosphorus plus background colour of some samples.

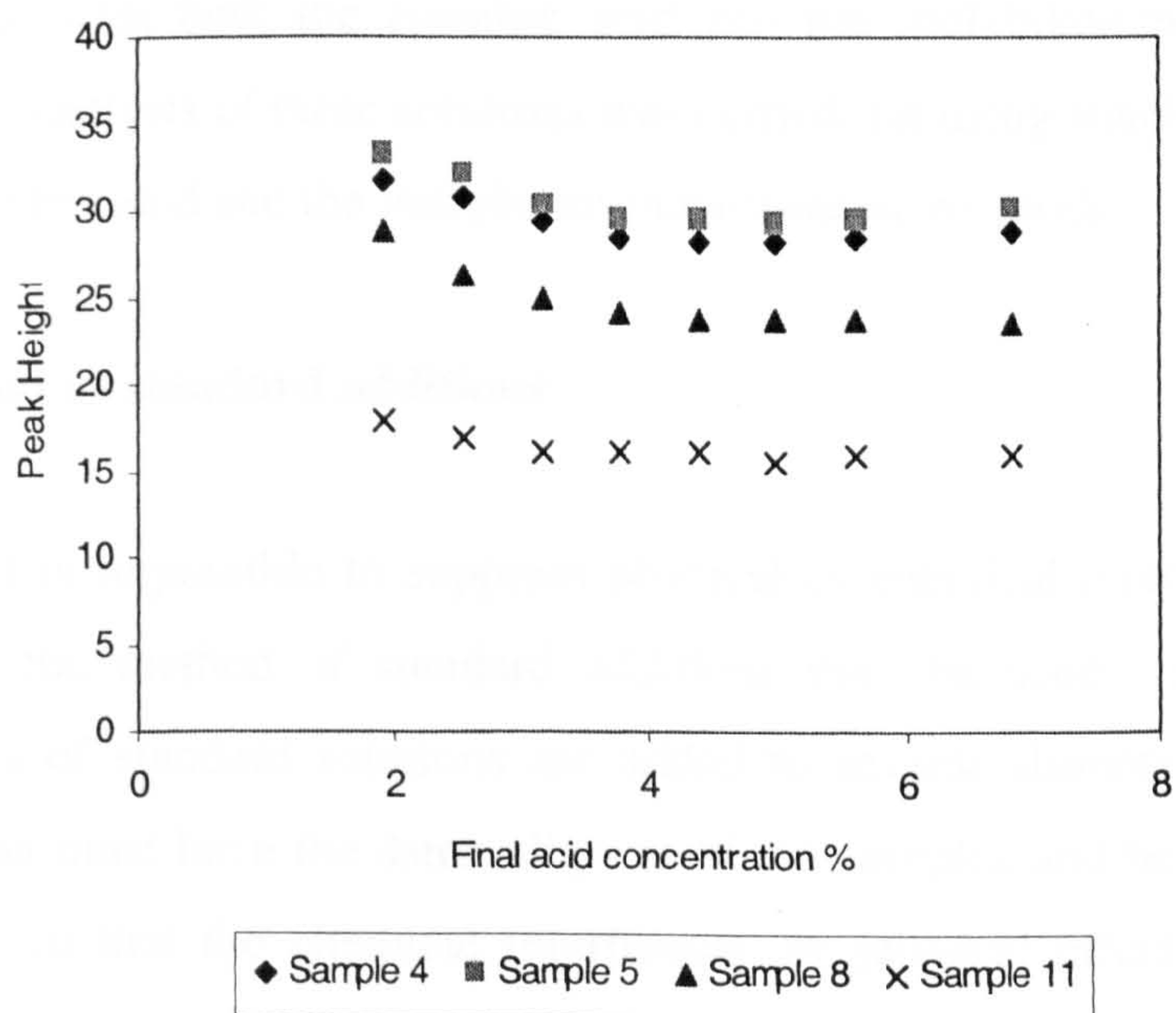


Figure 3.8: The effect of the final acid concentration on the phosphorus of some samples.



### **3.3 Chemical Problem in the Determination of Phosphorus with the Molybdate-Metavanadate Analysis Method in the Low Pressure Digest Solutions**

#### **3.3.1 Introduction**

Two certified plant materials along with a grass sample were digested with the low pressure digestion method as described in section 3.4.3.2. They then were analyzed for total phosphorus with both the ascorbic acid and the molybdate-metavanadate methods as described in section 3.4.4.1 and 3.4.4.2 respectively. There was a problem during analysis of digest solutions with the molybdate-metavanadate method. The results showed that the values with the molybdate-metavanadate analysis method were almost half of the values obtained with the ascorbic acid analysis method. Therefore it was decided to carry out a standard addition method to find out if there is a chemical interference problem with this analysis method for the low pressure digest solutions.

In order to compare the results these plants were also digested with concentrated perchloric acid as described in section 3.4.3.3. Both sets of digest solutions were analyzed with both the ascorbic acid and the molybdate-metavanadate methods. Another analysis of these solutions was carried out using standard addition with both the ascorbic acid and the molybdate-metavanadate methods.

#### **- Method of standard additions**

When it is impossible to suppress physical or chemical interference in the sample matrix, the method of standard additions may be used. In this method small volumes of standard solutions are added to several aliquots of the samples. All solutions must have the same aliquots of the samples and be made up to the same volume so that the chemical interference or physical effect is identical for each solution.



3.3.2 Materials and Methods

3.3.2.1 Certified Hay Powder

Certified reference material No. BCR 129 (Hay powder) with identification number 0088 supplied by the commission of the European Community Bureau of Reference was purchased from the office of reference Materials Laboratory of the government Chemist, Queens Road, Teddington, Middlesex TW11 0LY (table 3.4).

CRM	Element	Certified value %	Uncertainty half width of the 95% CI
BRC CRM 129 Hay Powder	P	0.236	0.006

Table 3.4: Certified value for total phosphorus in hay powder based on dry mass expressed.

3.3.2.2 Certified Cabbage Powder

Certified reference material No. GBW 08504 (cabbage powder) supplied by the Food Detection Science Institute Ministry of Commerce, Beijing, People’s Republic of China was obtained from the laboratory of the government Chemist (table 3.5).

CRM	Element	Certified value %	Uncertainty half width of the 95% CI
GBW 08504 Cabbage Powder	P	0.34	0.02

Table 3.5: Certified value for total phosphorus in cabbage powder based on dry mass expressed.

### **3.3.2.3 Grass**

The grass was collected from a field. It was dried at 80°C for forty eight hours and then ground with Glen Creston Mill, 14680.

### **3.3.2.4 Reagents**

**- Phosphorus working standard solutions: 0.0, 50, 100, 150, and 200mg/l phosphate-P**

They were prepared by dilution of stock solution in 5% perchloric acid and 12% mixed acid (2 volumes perchloric acid and 4 volumes nitric acid).

### **3.3.2.5 Procedure**

1– A volume of 10 ml digestion solution was pipetted into five 25ml glass vials.

2- 2 ml of each standard solutions (0, 50, 100, 150, and 200 mg/l phosphate-P) were added to the digestion solutions in the 5 glass vials and mixed well.

Phosphorus was determined using a Technicon Auto Analyzer II with both the ascorbic acid and the molybdate-metavanadate methods.

### **3.3.2.6 Calculation of Results**

The data can be plotted as a graph in which the concentration of standards added to the samples is on x axis and instrumental response is on y axis. The concentrations of phosphorus in the samples can be obtained graphically by extrapolating the line back onto the concentration axis. Figure 3.9 shows the graphic representation of this method (Willazd et al., 1988).



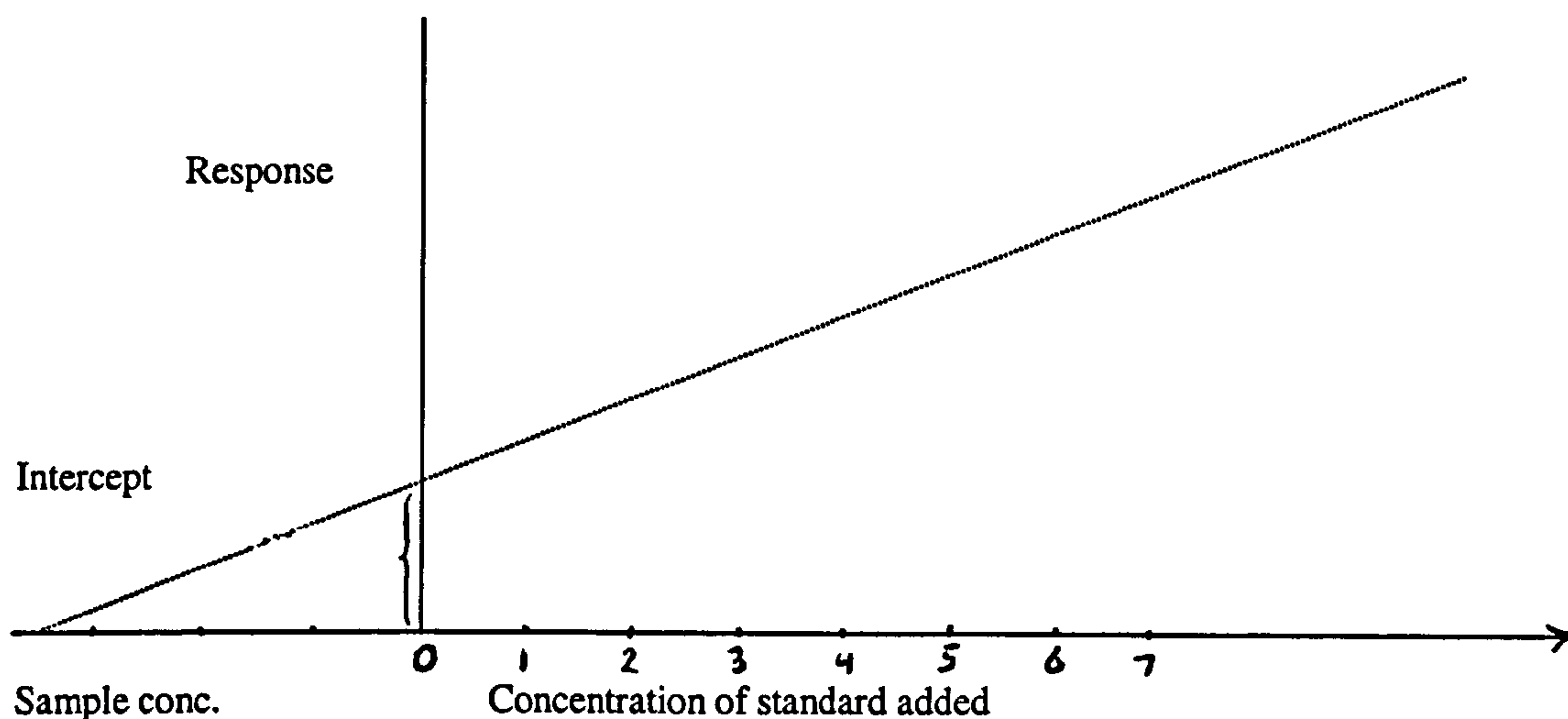


Figure 3.9: Graphic representation of the standard addition method of evaluation

$$\text{Slope} = \text{Intercept} / \text{Concentration of digest solution}$$

$\Rightarrow$

$$\text{Concentration of digest} = \text{Intercept} / \text{Slope}$$

The concentrations of phosphorus in the samples were also calculated from the equation obtained by linear regression.

As calculated concentration of phosphorus in the digest is in 12 ml solution or (10 ml digest +2 ml standard), the actual concentration of phosphorus in digest is

$$= \frac{\text{Intercept}}{\text{Slope}} \times \frac{6}{5}$$

### 3.3.3 Results and Discussion

Tables 3.6 and 3.7 show the values for three plant materials (two certified reference materials and one sample of grass), which were digested with two different digestion methods and were analyzed with two colorimetric methods (the ascorbic acid and the molybdate-metavanadate methods). The statistical analysis of t-test was applied to these data with the Minitab 13.1 package in order to obtain the 95% confidence interval of the mean of the results. Results were compared with the certified values



by looking at the overlap of the confidence intervals of the sample means with the confidence intervals of the certified values. This is less conservative than considering the overlap of confidence intervals of the sample means with the certified values.

Plant Material	Perchloric acid Digestion Method		Low pressure Digestion Method	
	Ascorbic Acid Method	Vanadate Method	Ascorbic Acid Method	Vanadate Method
Certified Hay	0.233 <sub>NS</sub> a	0.233 <sub>NS</sub> a	0.166 b*	0.089 * c
Certified Cabbage	0.345 <sub>NS</sub> a	0.349 <sub>NS</sub> a	0.244 b*	0.135 * c
Grass	0.437 a	0.436 a	0.398 b	0.204 c

Table 3.6: The % of phosphate-P in the three plant materials with the two digestion methods and two analysis methods with the normal calibration.

Certified Values: Hay 0.236 %± 0.006ψ. Cabbage 0.34 %± 0.02 ψ.  
 ψ: Half width of 95% confidence interval.  
 NS: 95% non significant difference from the certified values. p>0.05.  
 \*: Significant difference from the certified values. p< 0.05.  
 Numbers with the same letters in row are not significantly different (Fisher LSD test), p> 0.05.

The values obtained with the perchloric acid method are not significantly different from the certified values while the results obtained with the low pressure digestion method are all significantly less than certified values.

In order to compare the results of each plant digested with two digestion methods and analyzed with two analysis methods the Fisher LSD test was applied with the Minitab 13.1 package.

The values show that for the perchloric acid digestion of all 3 plant materials the results were not significantly different between the ascorbic acid and the molybdate-metavanadate method. The values also indicate that the phosphorus determined in the low pressure digest by the molybdate-metavanadate method are not in agreement with the ascorbic acid method. The molybdate-metavanadate values are almost half of the ascorbic acid values. The results obtained with the low pressure digestion method are significantly less than those obtained with the perchloric acid digestion method.

Plant Material	Perchloric acid		Low pressure	
	Ascorbic Acid Method	Vanadate Method	Ascorbic Acid Method	Vanadate Method
Certified Hay	0.234 NS	0.241 NS	0.171 *	0.165 *
Certified Cabbage	0.372 *	0.374 *	0.267 *	0.265 *
Grass	0.412	0.420	0.398	0.402

Table 3.7: The % of phosphate-P in the three plant materials with two digestion methods and two analysis methods with the standard addition calibration.

Certified Values: Hay 0.236 %± 0.006ψ. Cabbage 0.34 %± 0.02 ψ.

ψ: Half width of 95% confidence interval.

NS: 95% non significant difference from the certified values. p> 0.05.

\*: Significant difference from the certified values. p< 0.05.

Table 3.7 shows the values for these determinations obtained with the standard addition method. Because there were no replicates for these samples the results were compared with the certified values by looking at the overlap of the sample results with the confidence intervals of the certified values. No Fisher LSD test was used for these data.

The overall results confirm that the low pressure digestion method has a low recovery of phosphorus from the plant materials and that the ascorbic acid method produces a valid result. More over the molybdate-metavanadate method of analysis

gives lower values compared with the ascorbic acid method, which implies that a chemical interference happened during the analysis of these solutions with the molybdate-metavanadate method in normal calibration. Therefore this analysis method is not suitable for these digest solutions and ascorbic acid analysis method is a reliable method. However standard addition is far too complex and time consuming. Thus ascorbic acid method is a suitable analysis method for routine use.



## **3.4 Evaluation of Different Plant Digestion Methods for Determination of Phosphate-P**

### **3.4.1 Introduction**

Chemical analysis of plant material is performed for several different purposes such as foliar diagnosis, evaluation of the nutrient content of the crop, evaluation of the quality of feed stuff for bio industry and control of the agriculture products, etc (Houba et al., 1992). In order to better understand the nutrient availability in the growth medium, the analysis of leaf and plant tissue is necessary (Mengel and Kirkby, 1978). Plant analysis helps soil analysis to give a better answer for fertiliser needs in agriculture, the heavy metals and contaminated materials for national and international health purposes and regulations (Houba et al., 1992).

Chemical methods for the determination of total phosphorus in the plant material usually are performed through a two-stage procedure:

First: Phosphorus is converted to the orthophosphate form.

Second: The amount of the orthophosphate, which was produced, is determined by an appropriate analytical method (Olson and Summers, 1982).

#### **3.4.1.1 Digestion**

For the first stage there are several different methods to convert the plant phosphorus to orthophosphate. Dry ashing and wet acid digestion are the two main procedures for this purpose (Olsen and Summers, 1982) and (Dick and Tabatabai, 1982).

**A- Dry combustion method:**

In dry combustion method the organic matter of plant material is converted to ash by dry combustion and the dry soluble material in the ash is dissolved in an acid usually hydrochloric or nitric acid (MAFF/ADAS, 1986). Smith (1979) reported that total-P values obtained by dry ashing of plant sample were lower than those obtained with a

wet digestion. He pointed out that a single acid can yield higher metal, P and K than the dry ashing method. Van Lierop (1976) concluded the wet digestion method was more advantageous compared with dry ashing method. Anderson and Henderson (1988) considered the disadvantages of dry ashing method are the large quantity of sample requirement and also loss of some elements by volatilisation.

Smith (1979) pointed out that in dry ashing method substantial losses of potassium might happen at the temperatures higher than 450°C. Adrian (1973) mentioned the dry method is an acceptable method except when dealing with volatile metals.

#### B- Wet digestion method:

In the wet digestion method the plant material is oxidised with different single or mixed acids. These methods often rely on heating of the sample with concentrated acid (MAFF/ADAS, 1986). Wet digestion can be performed in two different ways:

- Digestion with a concentrated acid or mixture of acids in open system with conventional or microwave heating.
- Digestion with a concentrated acid or mixture of acids in closed (pressurised) system with conventional or microwave heating (Metajovic and Durackova, 1994).

Different investigators used wet methods, with a variety of single acids or mixtures of acids, temperature, digestion time, etc.

Turner and Brooks (1992) used nitric acid for total plant elements including phosphorus. Nitric-perchloric acid digestions of plant are often used for total plant cation determination (Ganje and Page, 1974). Several researchers such as Sommers and Nelson (1972) and Adrian (1973), have used this method for total plant phosphorus. The later used different ratios of nitric and perchloric acids. Glaubig and Poth (1993) used a time sequence study of nitric-perchloric digest for total-P and compared it with a Kjeldahl method.

Total digestion with a single acid or mixture of acids in a closed pressurised system with conventional or microwave heating was proposed (Metajovic and Durackova, 1994). In recent years the complete digestion of environmental, biological material by microwave was proposed (Metajovic and Durackova, 1994), (Soon and Kalra, 1995) and (Feng et al., 1999). The microwave method usually applies a pressure



digestion at 160-170°C up to 180Pa/i<sup>2</sup>. The closed system does not allow the use of perchloric acid due to the exothermic reaction with organic substances. However the higher pressure can significantly increase the degree and the speed of sample decomposition (Matejovic and Durackova, 1994).

Kjeldahl methods with different catalysts and different temperatures were used in different studies. There are also methods using H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> for total phosphorus.

Table 3.8 shows some different plant digestion methods used by different authors.

Amin (1995) used Kjeldahl method for plant-P analysis for certified plant materials in this department. He obtained the plant-P values higher than the certified values. The objective of this study was to evaluate the Kjeldahl method and other digestion methods with different temperature and heating systems using certified and other plant materials. Seven digestion methods were chosen in this study, which are explained in section 3.3.3.

- Three nitric acid digestion methods were used with different heating systems including a hotplate or digestion block at 120°C and high pressure in Teflon pressure vessel at 160°C.

A pressure digestion method was suggested in MAFF/ADAS book (1986) for soil and plant material in 150-160°C with nitric acid. Since the choice of digestion methodology is also a function of equipment availability, the high pressure digestion method was used in a sealed vessel at 160°C in an oven instead of microwave digestion method in this study.

- A nitric-perchloric acid digestion method, which is used with mixture of two acids, was also used as a low pressure digestion method at 60-70°C.

- Another digestion method, which is used in this study, is another nitric-perchloric digestion method with individual addition of nitric-perchloric acids at the temperature up to 180°C (MAFF/ADAS, 1986).

- Aqua Regia digestion method, which is usually used, for hard organic material and soils was included in the methods used in this study.



- Kjeldahl digestion method is also used with 2.5g catalyst and 375°C for plant-P determination.

Digestion Method	Procedure	Temperature°C	Author
Low Pressure Method	0.5 g plant +2ml HClO <sub>4</sub> +4ml HNO <sub>3</sub>	60-70 Sealed bottle	Adrian, 1973.
Low Pressure Method	0.5 g plant +2ml HClO <sub>4</sub> -H <sub>2</sub> SO <sub>4</sub> (7+1)+4ml HNO <sub>3</sub>	60-70 Sealed bottle	Adrian, 1973.
Low Pressure Method	0.1-0.3g plant+9MHClO <sub>4</sub> + 30% H <sub>2</sub> O <sub>2</sub>	80	Anderson, 1988
(Single Acid) HNO <sub>3</sub>	0.1 g plant + 1ml HNO <sub>3</sub>	130	Turner, 1992.
Mixed Acid	0.5g plant + 2ml HClO <sub>4</sub> -H <sub>2</sub> SO <sub>4</sub> (7+1) +4ml HNO <sub>3</sub>	Boiling	Adrian, 1973.
Mixed Acid	0.5g plant + 1ml HClO <sub>4</sub> -H <sub>2</sub> SO <sub>4</sub> (7+1) +2ml HNO <sub>3</sub>	Boiling	Adrian, 1973.
Mixed Acids	0.5g plant +11ml (8HNO <sub>3</sub> + 2H <sub>2</sub> SO <sub>4</sub> + 1HClO <sub>4</sub> )		Matejovic,and Durackova, 1994.

Table 3.8: Some plant digestion methods. The table is continued.....

Digestion Method	Procedure	Temperature°C	Author
HNO <sub>3</sub> / HClO <sub>4</sub>	0.133g plant + 1.33ml (HNO <sub>3</sub> / HClO <sub>4</sub> , 2:1)	120	Glaubig, 1993.
HNO <sub>3</sub> / HClO <sub>4</sub>	2g plant +30ml digest acid (HClO <sub>4</sub> +HNO <sub>3</sub> , 1:4), evaporate to dryness and redissolve in 2M HCl.	180-200	MAFF/ADAS, 1986.
HNO <sub>3</sub> / HClO <sub>4</sub>	0.2g sample + 3ml HNO <sub>3</sub> + HClO <sub>4</sub>	203	Summers, 1972
HNO <sub>3</sub> / HClO <sub>4</sub>	1g plant + 3.5ml HNO <sub>3</sub> +1.5ml HClO <sub>4</sub>	200	Zasoski, 1977.
HNO <sub>3</sub> / HClO <sub>4</sub>	0.5g plant		Raun, 1987.
Kjeldahl	0.15g plant +4ml H <sub>2</sub> SO <sub>4</sub> + 1g (Na <sub>2</sub> SO <sub>4</sub> : CuSO <sub>4</sub> , 5H <sub>2</sub> O, 10:1)		Hu, and Baker.1999.
Kjeldahl	0.2g plant + 5ml digest solution (100g K <sub>2</sub> SO <sub>4</sub> +1g Se in 1 litre H <sub>2</sub> SO <sub>4</sub> )		Twine, 1971.

The table is continued.....



Digestion Method	Procedure	Temperature°C	Author
Kjeldahl	2g plant +15ml 18MH <sub>2</sub> SO <sub>4</sub> +2.8g catalyst (Se: Na <sub>2</sub> SO <sub>4</sub> , 1:300)		Lyons, et al. 1985.
Kjeldahl	0.4g plant +10g catalyst (K <sub>2</sub> SO <sub>4</sub> : CuSO <sub>4</sub> , 5H <sub>2</sub> O, 100:1)		Thomas, 1967.
Kjeldahl	Digestion solution (350ml H <sub>2</sub> O <sub>2</sub> +420ml H <sub>2</sub> SO <sub>4</sub> + 14g Li + 0.42g Se).		Parkinson,1973.
Kjeldahl	0.1g plant +2.5ml H <sub>2</sub> SO <sub>4</sub> + H <sub>2</sub> SeO <sub>3</sub> +H <sub>2</sub> O <sub>2</sub>	380	Soon, 1995.
Kjeldahl	0.1g plant +2.5ml H <sub>2</sub> SO <sub>4</sub> +1.5gK <sub>2</sub> SO <sub>4</sub> +0.1g CuSO <sub>4</sub>	380	Soon, 1995.
Kjeldahl	0.1g plant +2.5ml H <sub>2</sub> SO <sub>4</sub> +1g (Anhydrous Na <sub>2</sub> SO <sub>4</sub> :Se, 100:1)	380	Soon, 1995.
Kjeldahl	0.1g plant +2.5ml (43.64g H <sub>2</sub> SeO <sub>3</sub> dissolved in 1 litre H <sub>2</sub> SO <sub>4</sub> )	380	Soon, 1995.

The table is continued.....

Digestion Method	Procedure	Temperature°C	Author
H <sub>2</sub> SO <sub>4</sub> - H <sub>2</sub> O <sub>2</sub>	0.250g plant + 5ml H <sub>2</sub> SO <sub>4</sub> +4drops %30 H <sub>2</sub> O <sub>2</sub> then, heat with 12 additions of H <sub>2</sub> O <sub>2</sub> .	225	Thomas, 1967.
H <sub>2</sub> SO <sub>4</sub> - H <sub>2</sub> O <sub>2</sub>	0.5g plant + 5ml H <sub>2</sub> SO <sub>4</sub> +drop H <sub>2</sub> O <sub>2</sub> until clear	Boiling	Adrian, 1973.
H <sub>2</sub> SO <sub>4</sub> - H <sub>2</sub> O <sub>2</sub>	0.25g plant +20ml H <sub>2</sub> SO <sub>4</sub> +3-5ml %30 H <sub>2</sub> O <sub>2</sub> +2ml HClO <sub>4</sub> .	Boiling	Van Lierop, 1976.
H <sub>2</sub> SO <sub>4</sub> - H <sub>2</sub> O <sub>2</sub>	Digestion solution (350ml H <sub>2</sub> O <sub>2</sub> +420ml H <sub>2</sub> SO <sub>4</sub> + 14g Li + 0.42g Se).		Parkinson, 1975.
H <sub>2</sub> SO <sub>4</sub> - H <sub>2</sub> O <sub>2</sub>	0.4mg plant + 4.4ml digest solution (350ml %30 H <sub>2</sub> O <sub>2</sub> + 0.42g Se +14g Li <sub>2</sub> SO <sub>4</sub> +420ml H <sub>2</sub> SO <sub>4</sub> )	350	Turner, 1992.
Sodium Hypobromite Oxidation	0.1mg plant +10ml sodium hypobromite, dissolve the dry residue in 5ml %18 formic acid +25ml N H <sub>2</sub> SO <sub>4</sub> ,	260-280	Dick and Tabatabai, 1982.
Dry Ashing	2g plant +dissolve the dry residue in 10 ml 6M HCl, + 2ml concentrated HCl, after second dry	500	MAFF/ADAS, 1986.

The table is continued.....

Digestion Method	Procedure	Temperature°C	Author
Dry Ashing	0.1g plant +0.5ml distilled water +1ml Mg(NO <sub>3</sub> ) 50% <i>m/v</i> + dissolve dry residue in 0.6N HCl.	550	Adrian, W.J. 1973.
Dry Ashing	0.5g plant +dissolve the dry residue in 5ml 5MHCl +0.5 ml HNO <sub>3</sub> , + 5ml 5MHCl after second dry.	500	Matejovic, & Durackova. 1994.
Dry Ashing	0.5g plant+ dissolve the dry residue in 2ml HNO <sub>3</sub> (conc.)	550	Adrian, 1973.
Microwave	0.5g plant+ 10ml HNO <sub>3</sub>		Matejovic, 1994.
Microwave	0.5g plant+ 10ml HNO <sub>3</sub> +2ml H <sub>2</sub> O <sub>2</sub>		Matejovic, 1994.
Microwave	0.5g plant +5ml HNO <sub>3</sub> +0.1 ml HF	175	Feng, 1999.
Microwave	0.5g plant + HNO <sub>3</sub> + H <sub>2</sub> O <sub>2</sub> + HCl	Sequential	Soon, 1995.



3.4.2 Materials

Two certified plant materials, beech leaves and bush twigs and leaves along with three non-certified plant materials: grass, lucerne and sycamore leaves were used to be digested with different digested methods. The description of each sample is as follows:

3.4.2.1 Beech Leaves

Certified reference material number CRM100 (beech leaves) prepared by the commission of the European Communities, Community Bureau of Reference was used.

The Table 3.9 comes from report number, EUR 12680 En and shows the amount of phosphorus in this certified material.

CRM	Element	Certified value %	Uncertainty half width of the 95% CI
BCR CRM 100 Beech Leaves	P	0.155	0.004

Table 3.9: Certified value for total phosphorus in Beech Leaves based on dry mass expressed.

3.4.2.2 Bush Twigs and Leaves

Certified reference material NO: GBW07603 (GSV-2), bush twigs and leaves prepared by the Institute of Geophysical and Geochemical Exploration, Lang Fang. Approved by state Bureau of Technical supervision, the People’s Republic of China (Table 3.10).

CRM	Element	Certified value %	Uncertainty half width of the 95% CI
GBW07603 Bush Twigs and Leaves	P	0.100	0.003

Table 3.10: Certified value for total phosphorus in bush twigs and leaves based on dry mass expressed.

**3.4.2.3 Grass**

The grass was collected from a field site, which had been fertilised with NPK fertiliser, before cultivation. It was dried at 80°C for forty eight hours and then ground with Glen Creston Mill, 14680.

**3.4.2.4 Lucerne**

Lucerne shoots were collected and dried at 80°C for forty eight hours and then ground with Glen Creston Mill, 14680.

**3.4.2.5 Sycamore Leaves and Twigs**

Sycamore leaves and twigs were collected. They were dried at 80°C for forty eight hours and then ground with Glen Creston Mill, 14680.

### **3.4.3 Digestion Methods**

#### **3.4.3.1 Plant Digestion with Nitric Acid (Hotplate)**

##### **Reagent**

- Concentrated nitric acid (AR), 69%.

##### **Procedure**

An amount of dried 1mm plant material equivalent to 0.2g oven dried basis was weighed into a 100ml conical flask. Very carefully 5ml concentrated nitric acid was added using a dispenser in a fume cupboard. The samples were covered with watchglasses, and stood overnight for predigestion. The samples were then placed on a hotplate and boiled gently for three hours at 120°C. After they were cooled, about 10ml deionized water was added to each digest, then they were transferred quantitatively to 50ml volumetric flask filtering through Whatman No: 50 filter papers. The conical flasks were washed two times with about 5ml deionized water and the washings were added to the filter papers. Finally the volumetric flasks were made up to volume with deionized water and mixed well.

Replication: 5 replicates of each plant material were digested.

Blanks: 5 blanks were carried out where no sample material was added to the digestion procedure.

#### **3.4.3.2 Plant Digestion with Low Pressure Method (Mixed Acid)**

##### **Reagents**

- Concentrated nitric acid (AR), 69%.
- Concentrated perchloric acid (AR), %60.



The mixture of two concentrated acids was made in a 500 ml brown glass. This mixture was made with 2 volumes of the concentrated perchloric acid plus 4 volume of the concentrated nitric acid.

### **Procedure**

Approximately 0.5g of dried 1mm plant material was weighed out accurately to four decimal places into a polypropylene screw cap bottle. Working in a fume cupboard very carefully 6ml of digestion acid was added. The bottle was capped loosely and left for predigestion overnight. Predigestion is essential because the bottle may burst if heated immediately. Then the bottles were capped tightly and placed in a water bath at 60-70°C for three hours. The top was removed and washed into the bottle with 2-3ml deionized water. The heating was continued for a further two hours to allow evaporation of the excess of volatile acid and then the bottles were removed from the water bath and allowed to cool. 10ml of deionized water was added and the digest was transferred qualitatively through a Whatman No. 50 filter paper to a 50ml volumetric flask washing with aliquots of deionized water.

Replication: 5 replicates of each plant were digested.

Blanks: 5 blanks were used when no sample material was added to digestion procedure.

### **3.4.3.3 Plant Digestion with Perchloric Acid**

#### **Reagents**

- Concentrated nitric acid (AR), 69%.
- Concentrated perchloric acid (AR), 60%.

#### **Procedure**

An amount of approximately 0.2g dried 1mm of each plant material was weighed out accurately (four decimal places) into a tall form Pyrex beaker. 10ml concentrated

nitric acid was added to each. The beakers were covered with a watchglass and allowed to stand for overnight predigestion. The samples were heated on a hotplate at approximately 100°C until brown fumes of nitrogen dioxide (NO<sub>2</sub>) were no longer evolved. The temperature was carefully increased until the acid was boiling gently. After 3 hours the watchglasses were removed and heating continued until the volume of each digest solution was reduced to approximately 5ml. The samples were removed from the hotplate and allowed to cool completely. Three ml concentrated perchloric acid were added and the digests were heated to just below the boiling point 180°C to evaporate the remaining nitric acid. When dense white fume of perchloric acid appeared, the beakers were covered with watchglasses and the heating was increased to a very gentle boiling, taking particular care that the digest do not boil dry, for two hours. The digests were cooled and 10-15ml deionized water added washing the watchglasses into the beakers. The digests were filtered through a Whatman No. 50 filter papers into 50ml volumetric flasks. The beakers were rinsed with at least three 10ml aliquots of deionized water, and added as separate aliquots into the filter papers, then the volume was made up to 50ml with deionized water and the solution mixed well.

Replication: 5 replicates of each plant material were digested.

Blanks: 5 blanks were carried out where no sample material was added to the digestion procedure.

#### **3.4.3.4 Plant Digestion with High Pressure Method**

##### **Reagent**

- Concentrated nitric acid (AR), 69%.

##### **Procedure**

An amount of 1mm dried plant material equivalent to 0.2g oven dried basis was weighed into a Teflon digestion container. Very carefully 5ml concentrated nitric



acid was added using a dispenser in a fume cupboard. The digest containers were capped loosely and left to predigest overnight.

An oven was adjusted to 160°C with an air extraction system inside it, to remove NO<sub>2</sub> fumes.

The digest containers were capped tightly and placed in the oven for 30 minutes. The oven was turned off and its door opened to cool and the digest containers removed. The tops were removed and washed into the container with 2-3ml deionized water for each top. 10ml deionized water were added and digest solutions were transferred quantitatively through a Whatman No. 50 filter paper to 50 ml volumetric flasks. They were then washed with aliquots of deionized water and made up to volume with deionized water.

Replication: 5 replicates of each plant material were digested.

Blanks: 5 blanks were carried out where no sample material was added to the digestion procedure

### **3.4.3.5 Plant Digestion with Digestion Block Method (Nitric Acid)**

#### **Reagent**

- Concentrated nitric acid (AR), 69%.

#### **Procedure**

An amount of dried plant material equivalent to 0.2g oven dried basis was exactly weighed using a four figures balance into an aluminium foil weighing boat. The plant sample was placed in the bottom of the 30cm digestion tube using long forceps. The weighing boat was weighed again to obtain the exact weight of sample digest in case a small amount of plant sample remained in it. 5 ml concentrated nitric acid was added to each tube using a dispenser in a fume cupboard. They were then stood overnight predigestion. The tubes were placed in the cool digestion block and the temperature was set at 120°C. The extraction unit was switched on to remove the brown NO<sub>2</sub> gas evolved during the digestion. The sample was digested



for 3 hours, until the tubes were clear of brown gas. The tubes were allowed to cool and 10ml deionized water was added to the digest which was transferred quantitatively to the 50ml volumetric flask through a Whatman No. 50 filter paper. The tubes were washed 3 times with deionized water and the washings added into the filter papers.

Finally the volumetric flasks were made up to volume with deionized water and mixed well.

Replication: 5 replicates of each plant material were digested.

Blank: 5 blanks were carried out where no sample material was added to the digestion procedure.

### **3.4.3.6 Plant Digestion with Aqua Regia Method**

#### **Reagent**

#### **Aqua Regia solution**

- 6 M hydrochloric acid.
- Concentrated nitric acid.

3 volume 6M hydrochloric acid was added to 1 volume of concentrated nitric (AR), 69% acid and mixed.

#### **Procedure**

An amount of approximately 0.2g of dried plant material was weighed out to four decimal places into an aluminium foil weighing boat. The plant sample was placed in the bottom of the 30cm digestion tube using long forceps. The weighing boat was weighed again to obtain the exact weight of sample digest in case a small amount of plant sample remained in it. 5ml of Aqua Regia solution were added to each and they were stood over night for predigestion. They were placed in a cool digestion block and heated at 125°C for 3 hours. They were cooled and removed from the digestion block. About 10ml deionized water was added to each and mixed. They

were then filtered quantitatively through Whatman No. 50 filter paper in to 50ml volumetric flasks. The tubes were washed 4 times with deionized water and the washings added into the filter papers.

Finally the volumetric flasks were made up to volume with deionized water, mixed well and remained to be analysed.

Replication: 5 replicates of each plant material were digested.

Blanks: 5 blanks were carried out where no sample material was added to the digestion procedure.

### **3.4.3.7 Plant Digestion with Kjeldahl Method**

#### **Reagents**

##### **- Kjeltabs**

Kjeltabs, which were used in this experiment, were purchased from Thompson and Capper Ltd. 3 Goddard Rd. Astmoos Industrial Estate, Runcorn, Cheshire WA7 1PH England.

Each tablet contains 5g of the mixture (100 parts  $K_2SO_4$  plus 6 parts  $CuSO_4$ , 5  $H_2O$  and 1 part selenium).

Selenium is a toxic substance and care should be taken while working with it in the fume cupboard.

##### **- Sulphuric acid**

Concentrated (AR) sulphuric acid 97% was used.

#### **Procedure**

An amount of approximately 0.2g dried plant material was weighed out accurately (four decimal places) into an aluminium foil weighing boat. The plant sample was placed in the bottom of the 30cm digestion tube using long forceps. The weighing boat was weighed again to obtain the exact weight of sample digest in case a small

amount of plant sample is remained in it. 2.5g catalyst salt mixture (half of a tablet) was put in each tube and 5ml concentrated sulphuric acid was added. The samples were left overnight for predigestion and then placed in a cold digestion block. The temperature was increased gradually until the digest cleared. It was then adjusted to 375°C. The heating was continued for another one hour.

The digestion tubes were cooled and removed from the digestion block. About 10 ml deionized water was added to each tube, mixed and cooled again. The digests were filtered quantitatively through Whatman No. 50 filter papers into 100ml volumetric flasks. The tubes were washed 3 times with deionized water, which was added to filter paper.

Finally the volumetric flasks were made up to volume with deionized water, mixed well and stored to be analysed.

Replication: 5 replicates of each plant material were digested.

Blanks: 5 blanks were carried out where no sample material was added to the digestion procedure.

### **3.4.4 Analysis Methods for Phosphate**

As was explained earlier in section 3.4.1 most plant phosphorus determinations have two different stages. The first stage is the digestion method and the second stage for this approach is the analytical method. There are different methods for analytical purpose. There are two colorimetric methods, which are widely used for plant phosphorus analysis. The concentration of phosphorus in the solution was determined by these two methods:

- The ascorbic acid-molybdate method.
- The molybdate-metavanadate method.

#### **3.4.4.1 Ascorbic Acid-Molybdate Method**

As described in section 2.4.8.



## **- Analysis of phosphorus in different matrices**

The diagram Figure 2.6 shows the general determination of phosphate-P. For plant digests with concentrated nitric acid, concentrated perchloric acid, concentrated mixed nitric and perchloric acid, low pressure, high pressure, block digestion, Aqua Regia and Kjeldahl, the sample solution had different concentrated acid. Therefore standard solutions, wash chamber and diluent were prepared in the appropriate matrix. For example, in plant digestion with concentrated nitric acid the final sample digest solutions had 10% nitric acid, therefore, standard solutions and wash chambers were prepared in 10% nitric acid (Table 3.11).

System Character	Digestion Methods						
	Nitric Acid	High Pressure	Digestion Block.	Low Pressure	Perchloric Acid	Kjeldahl	Aqua Regia
Matrix of Digest	10% HNO <sub>3</sub>	10% HNO <sub>3</sub>	10% HNO <sub>3</sub>	12% mixed acid	5% HClO <sub>4</sub>	5% H <sub>2</sub> SO <sub>4</sub>	10% Aqua Regia
Wash B	10% HNO <sub>3</sub>	10% HNO <sub>3</sub>	10% HNO <sub>3</sub>	12% mixed acid	5% HClO <sub>4</sub>	5% H <sub>2</sub> SO <sub>4</sub>	10% Aqua Regia
(mg/l)Highest Standard*	20	20	20	50	20	20	20
Diluent	10g/l NaOH	10g/l NaOH	10g/l NaOH	Water	Water	25g/l NaOH	5g/l NaOH
Dilution Ratio	4.75	4.75	4.75	13	4.75	4.75	4.75

Table 3.11: System characters for different digestion methods in the determination of phosphorus with the ascorbic acid analysis method.

\*: The matrices of the standard solutions are the same as the wash chamber B.

3.4.4.2 Molybdate-Metavanadate Method

Phosphate was determined on the Technicon AutoAnalyzer II as the yellow phospho-vanado-molybdate complex at 420nm in the range 0-50mg/l (Figure 3.9).

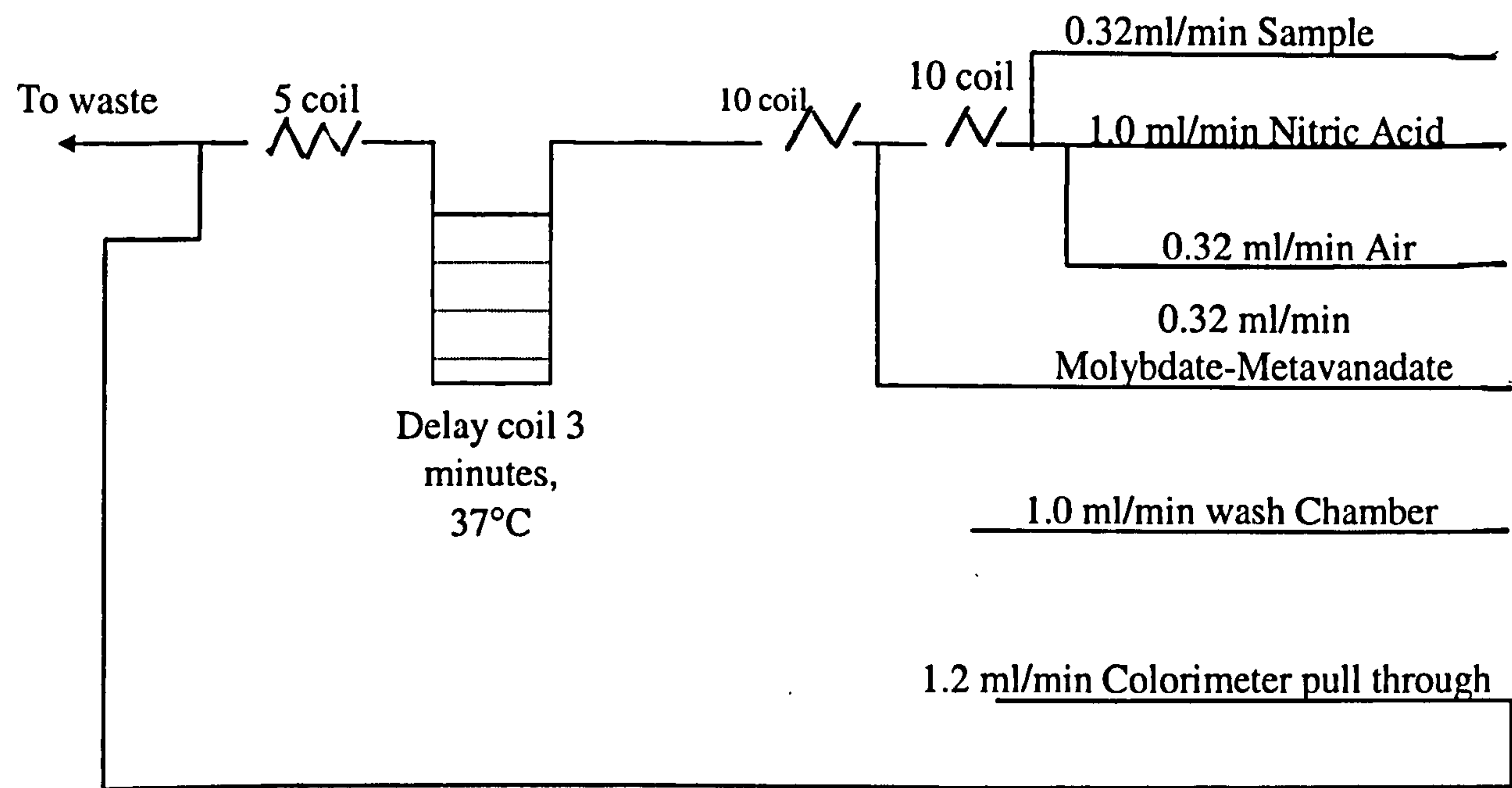


Figure 3.9: System manifold for phosphate-P analysis with the molybdate-metavanadate method.

- Reagents

Analytical grade reagents and deionized water were used throughout the experiment.

- Ammonium molybdate -ammonium metavanadate

25g of ammonium molybdate (AR) and 1.25 g of ammonium-metavanadate (AR) were dissolved in 90 ml of deionized water. The solution was made up to 1000 ml with deionized water and degassed.



**- Phosphate standard stock solution (1000mg/l)**

Phosphate standard stock solution (1000mg/l) was prepared as described in section 2.4.7.1

**- Phosphorus working standard solutions (0, 20, 40 and 50mg/l)**

Phosphorus working standard solution 0, 20, 40, and 50mg/l were prepared as by dilution of stock solution in appropriate metrics.

**- Wash chamber solution**

Wash chambers were also prepared in the appropriate acid solutions, as shown in the Table 3.12.

**- Nitric acid solution**

The nitric acid solutions were prepared in the concentrations appropriate to the different analysis system, which were run for the different digest solutions, provided that it maintains the 0.6M acid in the final coil in the analysis system.

System character	Digestion method						
	Nitric Acid	High Pressure	Digestion Block	Low Pressure	Perchloric Acid	Kjeldahl	Aqua Regia
Matrix of digest	10% HNO <sub>3</sub>	10% HNO <sub>3</sub>	10% HNO <sub>3</sub>	12% mixed acid	5% HClO <sub>4</sub>	5% H <sub>2</sub> SO <sub>4</sub>	10% Aqua Regia
Wash B	10% HNO <sub>3</sub>	10% HNO <sub>3</sub>	10% HNO <sub>3</sub>	12% mixed acid	5% HClO <sub>4</sub>	5% H <sub>2</sub> SO <sub>4</sub>	10% Aqua Regia
(mg/l) Highest Standard	20	20	20	50	20	20	20
HNO <sub>3</sub> Tube	3%+1ml/l Levor	3% +1ml/l Levor	3% +1ml/l Levor	3% +1ml/l Levor	5.2%+1ml/l Levor	water+1ml/l Levor	4.5% +1ml/l Levor

Table 3.12: Matrices concentration, wash B, nitric acid concentrations and standards for 7 digestion methods for the determination of the phosphorus by the Molybdate-Metavanadate analysis method.

### 3.4.5 Results and Discussion

The values of plant phosphorus obtained with seven digestion methods using five plant materials in this experiment are presented in Tables 3.13 and 3.14 and Figures 3.10 and 3.11. Table 3.13 gives the plant-P values obtained with the ascorbic acid method and Table 3.14 gives the results obtained with the molybdate-metavanadate analysis method.

Bonferroni based pairwise multiple comparisons was used to analyse the data using Minitab statistical software. Figure 3.11 shows the percentage of maximum values, which were obtained for P in the different plant materials with different digestion methods and two analysis methods. The values which are underlined or overlined within the same line are statistically not significantly different ( $p>0.05$ ).

The certified value for beech leaves is 0.155% P and for bush, twigs and leaves is 0.100% P. The results of different digestion methods for these two plants show that the perchloric and the Kjeldahl digestion methods have higher values (% of recovery) compared with other digestion methods. The values for the two certified plant materials with these two digestion methods are also in good agreement with each other and with the certified values. The digest solutions with the perchloric digestion method were completely clear and there was no colour interference in colorimetric determination of phosphorus by the molybdate-metavanadate and ascorbic acid methods.



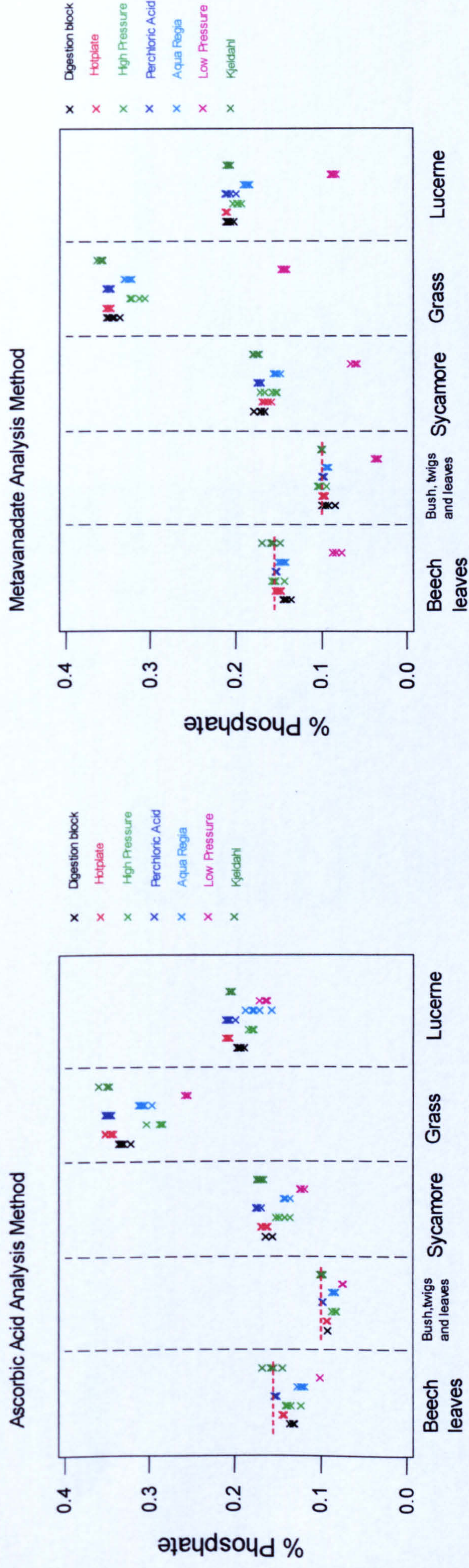


Figure 3.10: Plant-P with different methods.

- Dotted lines are the certified values.



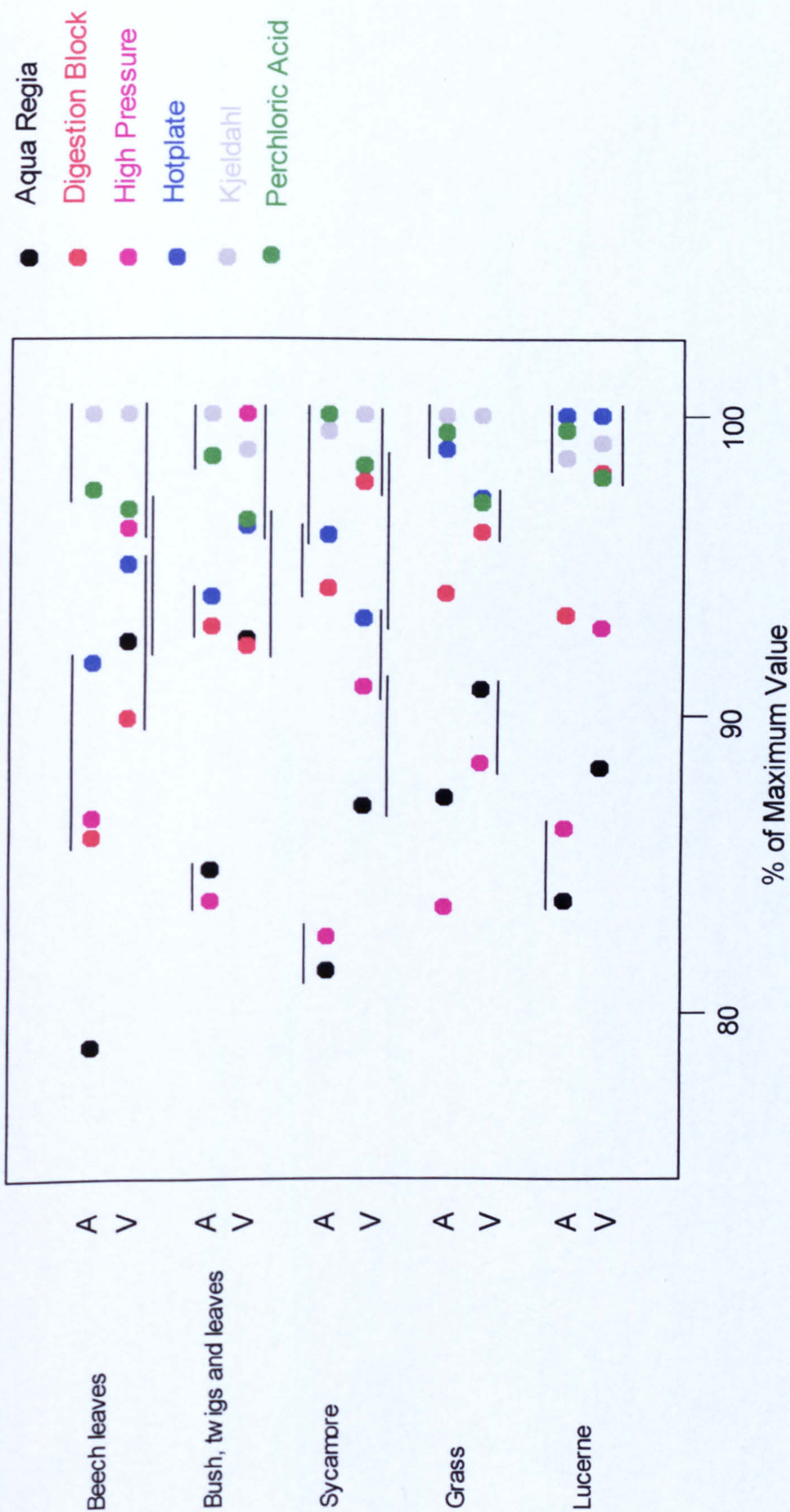


Figure 3.11: Percentage of maximum values with different digestion methods.

A: Ascorbic acid analysis method.

V: Metavanadate analysis method.

Values connected with a line are not significantly different ( $p > 0.05$ )



Digestion Method	Beech leaves	Bush, Twigs and leaves	Sycamore	Grass	Lucerne
	Plant –P (%)				
Certified Values	0.155% ±0.004 ψ	0.100% ±0.003 ψ			
Nitric acid(Digestion Block)	0.134 * b	0.092 * b	0.162 b	0.331 b	0.195 b
Nitric acid(Hotplate)	0.143 * b	0.093 * b	0.165 a b	0.348 a	0.209 a
Nitric acid(High Pressure)	0.135 * b	0.083 * c	0.142 c	0.294 d	0.181 c
Perchloric acid	0.152 NS a	0.098 NS a	0.172 a	0.350 a	0.208 a
Aqua Regia	0.123 * c	0.084 * c	0.140 c	0.307 c	0.175 c d
Low pressure	0.100 * d	0.074 * d	0.122 d	0.257 e	0.165 d
Kjeldahl	0.156 NS a	0.099 NS a	0.171 a	0.352 a	0.206 a

Table 3.13: The percentage of plant-P values with ascorbic acid analysis method.

ψ: Half width of 95% confidence Interval.

NS: 95% non significant difference from the certified values. P >0.05.

\*: Significant difference from the certified values. p< 0.05.

Numbers with the same letters in column are not significantly different (Fisher LSD test), p> 0.05.



Digestion Method	Beech leaves	Bush, Twigs and leaves	Sycamore	Grass	Lucerne
	of Plant –P (%) )				
Certified Values	0.155±0.004 %ψ	0.100±0.003 % ψ			
Nitric acid (Digestion Block)	0.142 * c	0.094 NS b	0.172 a b	0.346 b	0.208 a
Nitric acid (Hotplate)	0.149 NS b c	0.098 NS a b	0.165 b c	0.351 b	0.212 a
Nitric acid (High Pressure)	0.153 NS a b	0.101 NS a	0.160 c d	0.318 c	0.197 b
Perchloric acid	0.153 NS a b	0.099 NS a b	0.173 a b	0.350 b	0.208 a
Aqua Regia	0.146 * b c	0.094 * b	0.153 d	0.327 c	0.187 c
Low pressure	0.083 * d	0.035 * c	0.061 e	0.144 d	0.086 d
Kjeldahl	0.158 NS a	0.100 NS a	0.176 a	0.360 a	0.210 a

Table 3.14: The percentage of plant-P values with metavanadate analysis method.

ψ: Half width of 95% confidence Interval.

NS: 95% non significant difference from the certified values. P >0.05.

\*: Significant difference from the certified values. p< 0.05.

Numbers with the same letters in column are not significantly different (Fisher LSD test), p> 0.05

Due to the presence of  $\text{CuSO}_4$  as a catalyst in the Kjeldahl method there was a very slight blue colour which could potentially intensify the blue colour during the ascorbic acid analysis. An acceptable correction was carried out by subtracting the mean of the blanks from the sample peaks.

The results with the other plant materials also showed higher values with the perchloric and Kjeldahl digestion methods compared with the other digestion methods, which can be seen in Figure 3.11. The only exception is the grass, which gave a lower value with the molybdate-metavanadate method for the perchloric digest solution.

The results obtained for the two certified plant materials with the nitric acid digestion methods with different heating systems (the hotplate, the digestion block and the high pressure) are significantly lower than the certified values. They are also generally lower than those obtained with the perchloric acid and the Kjeldahl digestion methods.

The results obtained for the nitric acid digestions were also inconsistent between methods and plant materials. For example the value for the nitric acid with the hotplate heating digest in the certified beech leaves were significantly lower than those obtained with the perchloric and Kjeldahl. On the other hand the grass, lucerne and sycamore digested with hotplate heating and analysed by ascorbic acid and lucerne digested with digestion block and hotplate and analysed with molybdate-metavanadate gave results which were in agreement with highest values and not significantly different to the perchloric and Kjeldahl methods.

After two hours of digestion these digest solutions were still yellow. The colour correction was made for these samples i.e. the yellow colour was measured without colour development reagents and their peak heights were subtracted from the main peaks. However the difference obtained by ascorbic acid and molybdate-metavanadate methods raise questions about the success of the background colour correction.



In the high pressure digestion method there was some residue in the solution after the procedure was finished and digest solutions were also still yellow. The 30 minutes was probably not enough to equilibrate the temperature of outside and inside the vessels.

The plant-P results with the Aqua Regia method gave a lower value both with the ascorbic acid and the molybdate-metavanadate analysis methods for all plant materials. However the ascorbic acid values were less than the molybdate-metavanadate values for this digestion method. These digests were yellow at the end of the digestion. This again raises a question about the successful background colour correction.

When nitric-perchloric acid was used as a mixture of acids in the low pressure digestion method, there were two problems, firstly this digestion method had a low recovery and secondly there was a problem with the plant-P values obtained with the molybdate-metavanadate analysis method. This is shown clearly in Figure 3.10. The results obtained with the molybdate-metavanadate analysis method were almost half and significantly different from those obtained with the ascorbic acid analysis method ( $p < 0.05$ ). This problem is a chemical interference, which is discussed in section 3.3 in this study. However the values even with the ascorbic acid analysis method of this digest solutions were significantly less than certified values ( $p < 0.05$ ). They were also less than those obtained with the perchloric acid and the Kjeldahl digestion methods. The lower recovery of plant-P with this digestion method is probably because of incomplete breakdown of organic material due to the low temperature (60-70°C).

Glaubig and Poth (1993) obtained 3.4% higher plant-P using nitric-perchloric digestion compared with the Kjeldahl method. They recommended the nitric-perchloric acid digestion method as a suitable replacement for the Kjeldahl digestion method for plant-P analysis. They used the digestion acid as a mixture of two acids. As they used a time sequence study his recommended method is based on 16 hours digestion.



Sommers and Nelson (1972) suggest nitric-perchloric acid digestion at 203°C as a reasonable method for estimating total-P.

A sequential addition of nitric and perchloric acids was applied in the method used in this study. Firstly samples were heated covered with a watch glass with nitric acid at about 120°C to partially oxidise the organic matter. This was done to avoid any explosion of organic matter contacting perchloric acid. They were then heated uncovered for more digestion of the plant materials and to evaporate some of the nitric acid. The perchloric acid was then added to raise the temperature and complete the phosphate release. The samples were heated to evaporate the remaining nitric acid and covered for second time and heated at higher temperature of 180-200°C. The boiling point of nitric acid is less than perchloric acid 122°C compared with 203°C (Turner and Brooks, 1992) and (Analytical Committee, 1959).

Zasoski and Burau (1977) used the nitric-perchloric digestion method with the individual addition of acids for plant analysis with digestion at 80°C for the nitric acid and subsequently at 180°C for the perchloric acid. They suggested the method for high speed processing of samples and was particularly good for trace element and phosphorus analysis and an acceptable method for Ca, Mg, and K. A wet digestion with the nitric-perchloric acid is also suggested by MAFF/ADAS book (1986), for complete digestion of plant material. Anderson and Henderson (1988) and Zhao et al. (1994) also suggested that the nitric-perchloric acid is a reliable method for plant-P determination. Alder and Wilcox (1985) suggested the perchloric acid digestion as a reliable method for plant digestion and element analysis.

Smith (1979) found higher values for plant-P by mixed nitric-perchloric-sulphuric acid digestion compared with the peroxidation and dry ashing methods. He added that the differences between the methods were not consistent between elements or between tissue types, but the nitric-perchloric-sulphuric acid digestion invariably yielded higher cation values than the other methods. Anderson and Henderson (1988) obtained low values for potassium by using a nitric-perchloric acid digestion method. They attributed the lower recovery of potassium in this method to

precipitation of potassium perchlorate in this digestion procedure. Some researchers like Metajivic and Durackova (1994) obtained lower values for plant-P for the nitric-perchloric-sulphuric acids (8:2:1) digestion compared with the dry ashing method. However the nitric-perchloric digestion method, which was used in present study, obtained higher values for plant-P. This method proved to be a reliable method since the solutions were clear and its results were reproducible.

The values for phosphorus obtained with the Kjeldahl digestion method used in this study gave a good agreement between the results and certified values. Soon and Kalra (1995) compared four different Kjeldahl digestion methods and obtained lower results for plant-P with a Kjeldahl method using  $K_2SO_4 + CuSO_4$  as a catalyst. The higher recovery of tissue phosphorus in their study was with Kjeldahl digestion using catalyst containing Se. They also obtained lower results with ascorbic acid analysis method compared with molybdate-metavanadate analysis method when Se was used as a catalyst and suggested that Se concentration  $>50\text{mg/l}$  causes low results with ascorbic acid method. Parkinson and Allen (1975) used a Kjeldahl digestion method with Se and  $LiSO_4 \cdot H_2O$  as a catalyst for plant material. They compared the method with a nitric-perchloric-sulphuric acid digestion and obtained higher values with Kjeldahl digestion method. Then they analysed phosphorus with the molybdate-metavanadate method and attributed the higher recovery of phosphorus to enhancement of the phosphomolybdate colour, as the digestions were still yellow when stopped. Amin (1995) obtained higher values for plant-P with the Kjeldahl digestion method. He used a  $K_2SO_4 - CuSO_4 - Se$  catalyst salt mixture for the procedure and obtained significantly higher results than the certified values. However the Kjeldahl method used in the present study gave values for plant-P, which were not significantly different from the certified values ( $p > 0.05$ ).

Adrian (1973) developed the low pressure digestion method with nitric-perchloric acids at  $60-70^\circ\text{C}$  which was used in the present study. He tested several digestion methods including dry ashing, low pressure digestion with nitric-perchloric acids, low pressure digestion with nitric-perchloric-sulphuric acids, hotplate digestion with nitric-perchloric-sulphuric acids and sulphuric acid plus  $H_2O_2$  with different volumes of acids and compared them for plant-P determination. He claimed that the



low pressure digestion method gave a good recovery of plant-P. He analysed the phosphorus with the molybdate-metavanadate method, and obtained yellow coloured digest solutions but claimed that there was no interference of yellow colour with the molybdate-metavanadate analysis method. However he did not use certified plant material to compare the obtained results with certified values. The methods yielded inconsistent results with significant differences between the different methods and also with the same method using different volumes of acids. Despite his report that there was no interference of yellow colour, there might have been some effects in the analysis.

Anderson and Henderson (1988) used a low pressure sealed chamber digestion method with perchloric acid and  $\text{H}_2\text{O}_2$  at  $80^\circ\text{C}$  for the multielement analysis of eight certified reference materials and reported that the sealed chamber digestion is satisfactory and sometimes exceeded the standard values. He also reported that some residue remained in the vessels after digestion. He obtained similar results for plant-P with dry ashing, sealed chamber digestion and nitric-perchloric digestion methods. However in the low pressure digestion method used in present study the temperature was lower ( $60\text{-}70^\circ\text{C}$ ) and despite the above workers its recovery was lower than the certified values ( $p < 0.05$ ) which is probably because of incomplete breakdown of organic matter at the low temperature.

### **3.4.6 Conclusion**

From the results obtained with different digestion methods it can be concluded that the perchloric and the Kjeldahl digestion methods are able to digest the organic materials better than the other digestion methods examined in this study. The advantage of the nitric-perchloric digestion method is the production of clear colourless digest and low residue due to the high temperature and oxidation power.

Many investigators try to use a single digestion method for N, P and K and metal elements with different success. Kjeldahl digestion contains  $\text{K}_2\text{SO}_4$  or  $\text{Na}_2\text{SO}_4$  to raise the boiling point and Se or  $\text{CuSO}_4$  as a catalyst. It therefore has a potential of



error in the determination of phosphorus with the ascorbic acid method because of the light blue colour of the Kjeldahl digest solution where Cu is used. There are also probabilities of precipitation of some elements with the sulphate anion. Novozamsky et al. (1993) and Temminghoff et al. (1992) considered the use of  $\text{H}_2\text{SO}_4$  is not suitable in the mixed acid digestion of plants for multielement analysis. They wrote that the presence of  $\text{H}_2\text{SO}_4$  could cause co-precipitation of some elements, which eventually come to low recovery of some element such as Pb. Turner and Brooks (1992) also reported of precipitation of calcium in the digest with the sulphate anion.

Based on the above considerations it was decided to use the Kjeldahl method for the determination of nitrogen and the perchloric digestion method for phosphorus and heavy metals in this research study. Also as the ascorbic acid method was carried out without problems in this experiment it was chosen to be used for phosphorus analysis in the digest solutions.

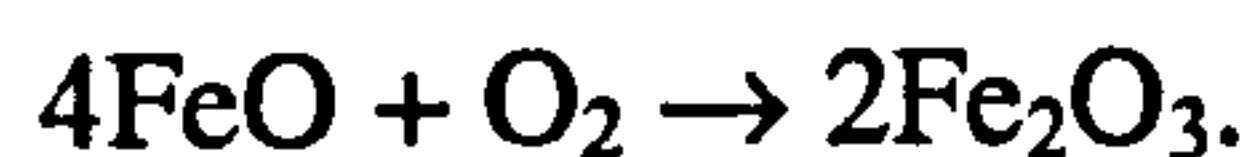
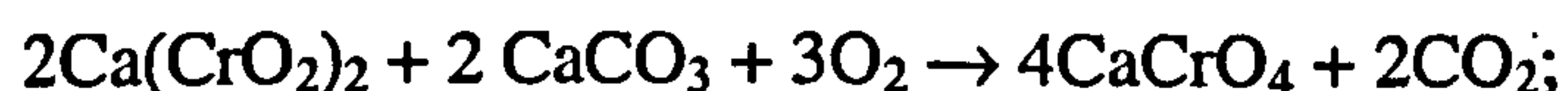
## CHAPTER FOUR

### A CASE STUDY OF A CHROMIUM CONTAMINATED LAND

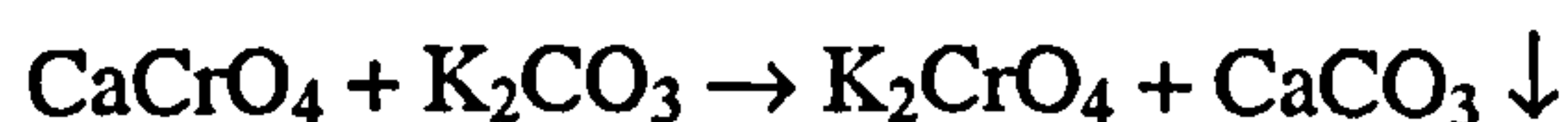
#### Survey

#### 4.1 Introduction - The History of the Contaminated Site

The site, which is called Summerford, is situated at the end of the Summerford Road, just off Windsor Road in Falkirk. The Ordnance Survey map Figure 4.1 shows the location of the site. This site had three main uses over the last 150 years. The 1859 map Figure 4.2 shows the site was occupied by a chemical works at the western end of the site. The works mainly dealt with the production of chromium salts. Potassium chromate and dichromate were the most important chromium compounds produced. They were produced from the chromium iron ore, chromite. In this process ore was finely ground and mixed with calcium carbonate which was then roasted in an oxidizing atmosphere. The roasted mixture was mixed with water. In the water mixture the calcium chromate went into solution and the ferric oxide remained behind. The probable reactions, which took place, were:



The calcium chromate solution was then mixed with potassium carbonate,



and the resulting solution of the potassium chromate was separated from the precipitated carbonate.

The works produced a calcium carbonate waste, which contained 1 to 3%  $\text{CrO}_3$ , which is equivalent of 5,000-15,000mg/kg Cr in  $\text{CaCO}_3$ . The manufacturing continued in operation until about 1930. During this time these wastes from the

chemical works were put on the area north of the main railway embankment. The Ordnance Survey map 1895-6 Figure 4.3 shows the location of the dump waste which was brought in by a branch railway line. There was also another branch railway coming from the Camelon Chemical Works, which may have brought some waste from this works to the area too. About 1910 an iron works was built on the north east corner of the site. The 1913 map Figure 4.4 shows new buildings and more spoil can be seen while on the 1944 map Figure 4.5 the chemical works and the associated railway has disappeared and the iron works can be seen. The iron works closed in about 1950 and a concrete firm named J.K. Miller moved to the area, but the 1950 map Figure 4.6 shows the concrete firm in the area formerly occupied by the iron works and the slag heaps at the eastern end of the spoil heap. In 1958 the map Figure 4.7 shows the loss of the railway sidings to the works. The 1971 map Figure 4.8 shows the location of the concrete firm, which was still in operation at that time.

In 1976 the precast concrete factory ceased trading and in 1977 restoration regrading work was started, when the Central Regional Council took over the site. It called in contractors to demolish the existing buildings. They then bulldozed them up against the side of the tip. The top of the tip was then leveled off by pushing the excess material over the top of the concrete waste rubble, which formed a fairly steep slope down to the flat area of the site. Therefore the site has three different parts. One part is located at the flat top of the tip, the second part is a slope area and the third is another flat part at the bottom of the slope. The photograph from 1991 Figure 4.9 shows the position of the site with the slope and with a flat part at the bottom of the slope and there would also be another flat part, which is located on the top of the site. In the picture a big bare part of the site can be seen.

After this preliminary work, developers showed interest in building houses on the land. The council sold two corners of the site for an old people's home and for private housing.

The poor vegetation and poor establishment of trees in the central part of the site on the spoil heap area led to the investigation of the area. The investigation showed pools of water with a yellow crystalline deposit with pH 9 and 60mg/l of soluble chromium going to the road and the storm drain carrying some sediment from the site.





Figure 4.9: The photograph of the site showing the position of different parts.



It also showed sediment samples in roadside dust with 3650mg/kg of total chromium and 1.8mg/l soluble chromium, and sediment samples in front of the old people home with 904mg/kg of total chromium and 0.8mg/l of soluble chromium. These findings showed chromium contamination, which necessitated remedial action to this site before any further development, could take place and to render the site safe. If the site is used for walking or games, then the bare soil could cause high chromium dust problems to occur in dry weather, which is toxic and carcinogenic. At that time there was no medical evidence covering the local population.

There were different suggestions made to overcome the problem, but they all were very expensive. Therefore cheaper solutions were sought to break the link between source of the contamination and the target. Fencing can be of limited benefit only because the children can still go easily and play in the area. Vegetation cover can reduce the runoff water and also reduce the dust in dry weather. Trees and bushes also can discourage access to the area. An efficient drainage system also could be useful and reduce the puddle of the runoff water containing chromium.

In 1992 sewage sludge was added to the flat part of the site at the bottom of the slope part to increase the organic matter and nutrients for better vegetation growth and a drainage system also was built in, which was drained to intercept runoff.

In order to understand the soil characteristic such as pH and soil organic matter, nutrient conditions in plant and soil, the total chromium and some other metals it was decided to carry out two different surveys across the site. One was a survey of vegetation and soil profiles with 18 sampling points.

The vegetation yield and soil nutrient level may vary during different times of the year due to temperature, rain fall or plant uptake, and possible oxidation of chromium. Another survey was intended to be done on the vegetation and 6 surface soils to see the seasonal pattern in plants and soils nutrients and chromium status.

## **4.2 Methods**

### **4.2.1 Sampling the Site**

18 sampling points were chosen from the whole site based on the three parts. Soil samples were taken from three depths 0-15cm, 15-30cm and 30-50cm (Figure 4.10). Some root material and the stubble and also some vegetation were sampled from each sampling point. The surface leaves and the branches were removed from the topsoil and all soil samples were put in plastic bags, labeled and carried to the laboratory. The three sampling points 1 to 3 were selected from the flat part of the top of the site, which had a good grass cover and some small birch trees to the back close to the railway embankment. The sampling point profiles had loose ash material. The sample number 2 had the carbonate waste at less than 50cm depth. Six sampling points 4 to 9 were chosen on the slope part. The sampling points 4, 5 and 6 were from the planted part west of the site. This side had a dense stand of small birch trees with some grass and the loose ash profile with many tree roots. The sampling points 7, 8 and 9 were located on the unplanted east side of the pathway on the slope area, where was very poorly vegetated with sparse birch trees. The profile contained loose ash with the calcium carbonate waste material from less than 30cm depth. Nine soil samples 10 to 18 were chosen from the flat part of the site at the bottom of the spoil, which had the patchy grass. The profile had uniform ash material with the very compact material bellow the 15cm depth. Some parts were very wet.

### **4.2.2 Sample Preparation**

The soil and the grass samples were prepared as described in the sections 2.1.1, and 2.1.2 respectively. The root materials were dried at room temperature and were shaken in order to remove the soil particles. They were then sieved and washed with the water and air dried again. They were then dried at 80°C for 48 hours and ground to pass through 2mm sieve.



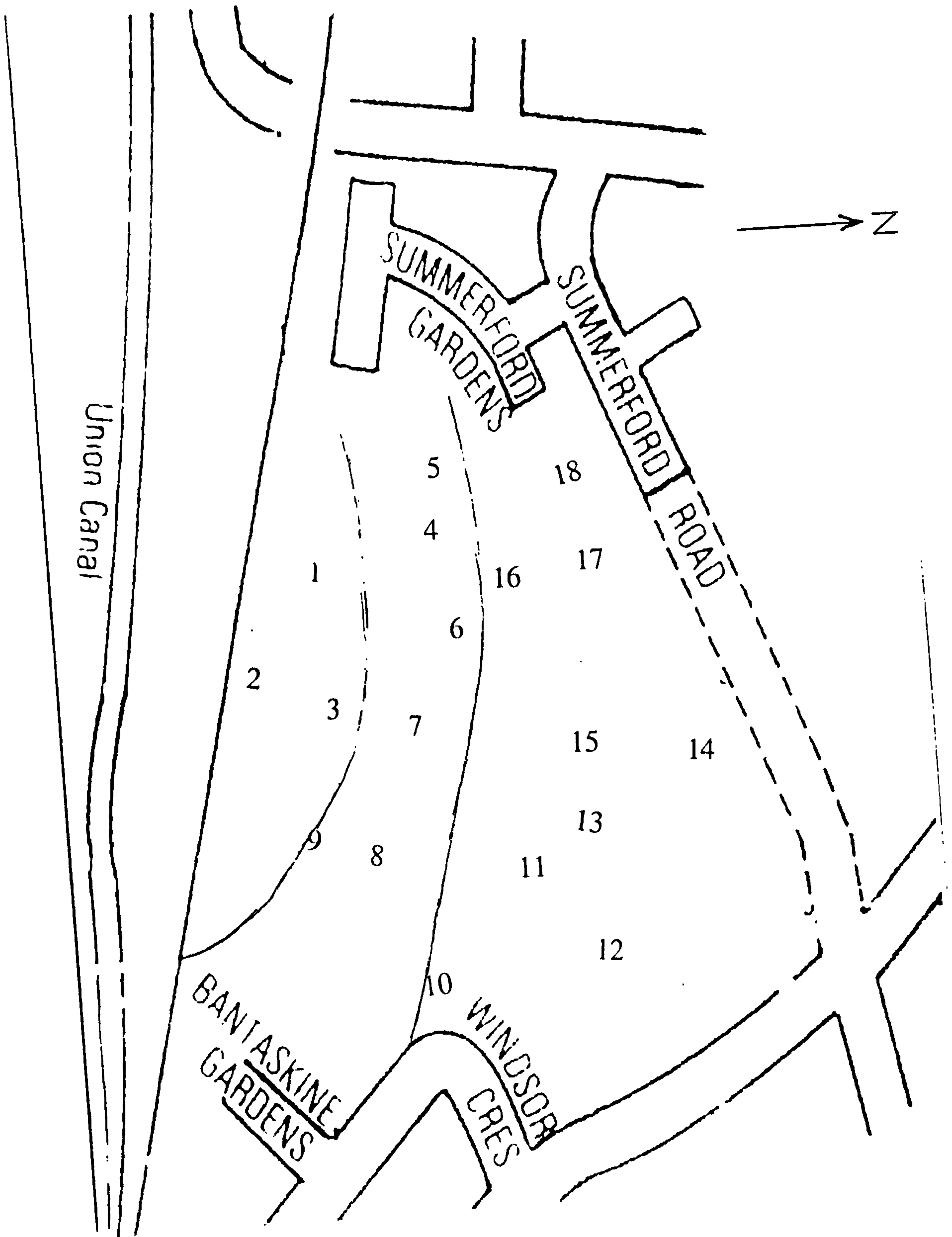


Figure 4.10: 18 sampling points for the first survey. Scale: 1/2500

### **4.2.3 The Analysis of Samples**

The pH and the percentage of the organic matter of the soils were measured as described in the section 2.1.3 and 2.1.9. The soils were extracted for inorganic nitrogen (ammonium-N, nitrite-N and nitrate-N), extractable phosphorus, extractable potassium, extractable magnesium and soluble chromium as described in the sections 2.2.1, 2.2.3, 2.2.4, 2.2.5 and 2.2.2 respectively. The soils and the plant material were digested for the total nitrogen as described in the sections 2.3.3 and 2.3.2 and the root mat as described in the section 2.3.2. The soils, the plant material and the root mat also were digested for the total P, K and metals as described in the section 2.3.1. The extractable ammonium-N, extractable nitrite-N and extractable nitrate-N, extractable P, extractable K and extractable Mg were measured as described in sections 2.4.3, 2.4.5, 2.4.6, 2.4.7, 2.4.9 and 2.4.2 respectively. The total P, N, K and metals were measured with the methods as described in the sections 2.4.8, 2.4.4, 2.4.10 and 2.4.1. The chromate, nitrite-N and nitrate-N in the water extract were measured as described in the section 2.4.11.

### **4.2.4 Seasonal Changes of the Site**

Six sampling points were chosen to represent the main sampling parts of the site. Soil samples were taken from the 0-15cm depth. The surface leaves and the branches were removed from the topsoil and all soil samples were put in plastic bags, labeled and carried to the laboratory. One square metre of vegetation was sampled from each sampling point. This was also put in plastic bags, labeled and carried to the laboratory along with the soil samples.

The soil and the grass samples were prepared as described in the sections 2.1.1, and 2.1.2 respectively.

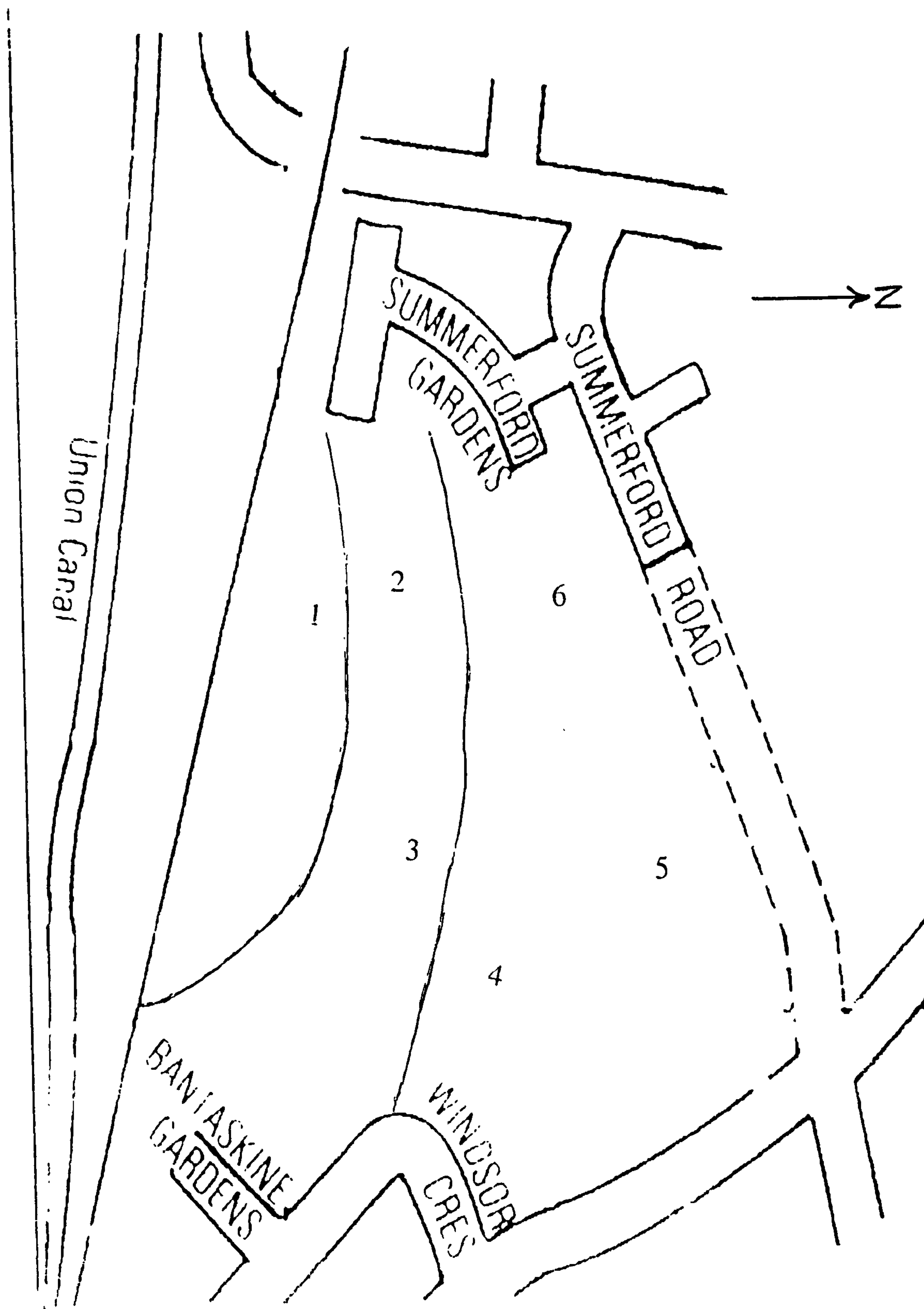


Figure 4.11: 6 sampling points for survey of the seasonal change. Scale: 1/2500



#### **4.2.4.1 The Analysis of Samples**

The soils were extracted for inorganic nitrogen (ammonium-N, nitrite-N and nitrate-N), extractable phosphorus, extractable potassium, extractable magnesium and soluble chromium as described in the sections 2.2.1, 2.2.3, 2.2.4, 2.2.5 and 2.2.2 respectively. The dry yield of plant material was measured as described in section 2.1.2. The plant material was digested for the total nitrogen as described in the sections 2.3.2. The plant material also was digested for the total P, K and metals as described in the section 2.3.1. The extractable ammonium-N, extractable nitrite-N and extractable nitrate-N, extractable P, extractable K and extractable Mg were measured as described in sections 2.4.3, 2.4.5, 2.4.6, 2.4.7, 2.4.9 and 2.4.2 respectively. The total P, N, K and metals were measured in the plant digests with the methods as described in the section 2.4.8, 2.4.4, 2.4.10 and 2.4.1.

### **4.3 Results and Discussion**

The results are presented in Tables 4.1 to 4.3 and Figures 4.12 to 4.26. The soil pHs (Figure 4.12) from the three different depths show high and similar values in the range of 7.8- 9.0. The exception is for the pH of the samples from the lower depth of sites, 7, 8 and 13 which is higher than the other soils. In the former industrial activity a large amount of waste material containing chromium and calcium carbonate was dumped in this site. The high pHs of the soil samples are probably because of this calcium carbonate.

Figure 4.13 shows the values for the organic matter in the soils. The percentage of the organic matter of these soils was determined by the potassium dichromate method as described in section 2.1.9. This was because the determination of the organic matter by LOI (Loss On Ignition) has the potential of overestimation the percentage of organic matter in soils containing large amounts of carbonate, due to the breakdown of carbonates. The figure shows variable values with a lack of increase in the top 15cm versus the next 15cm depth suggesting a lack of incorporation of organic matter into the soil profile.

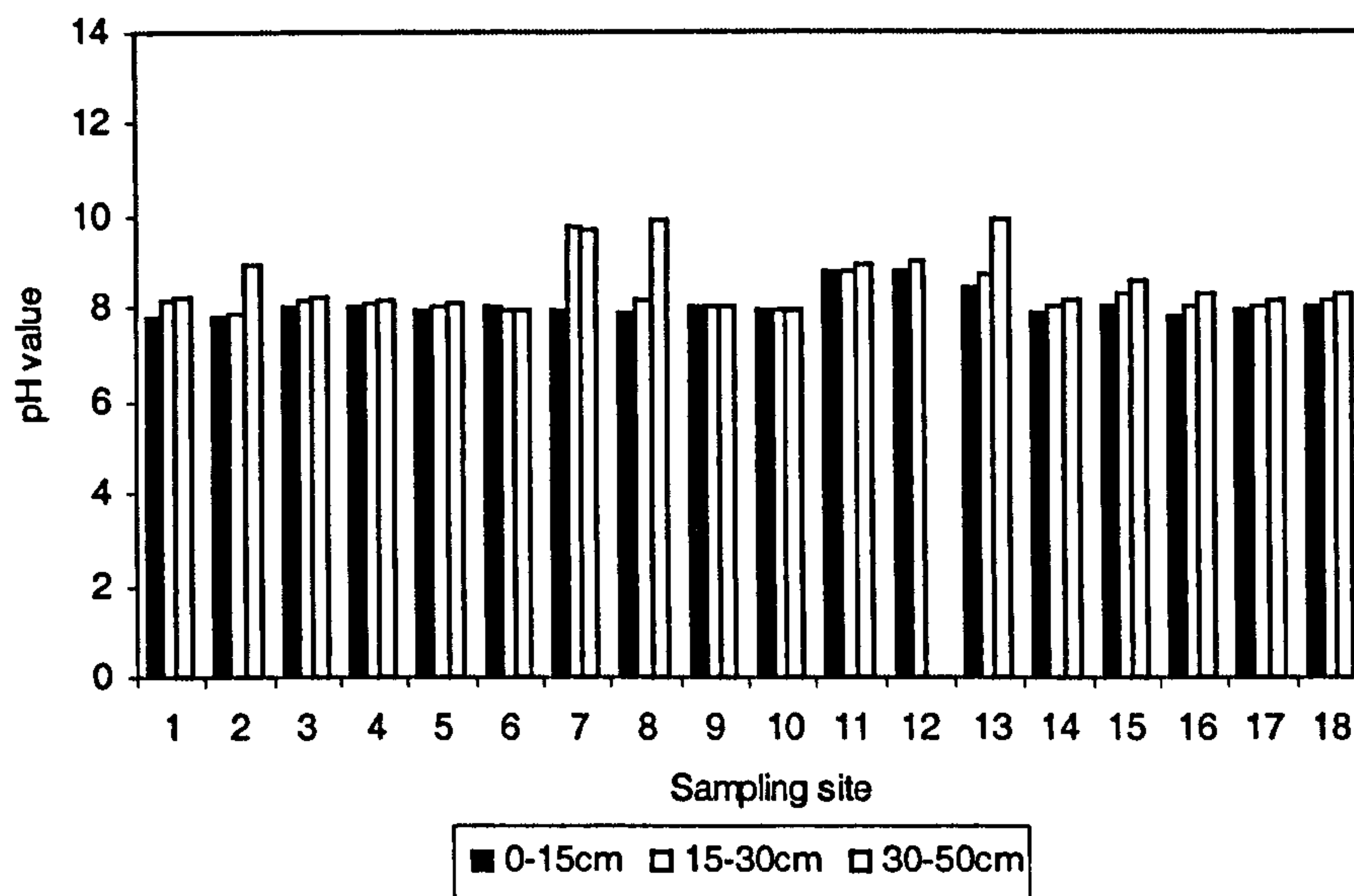


Figure 4.12: pH in soils from 3 different depths of 18 sampling points.

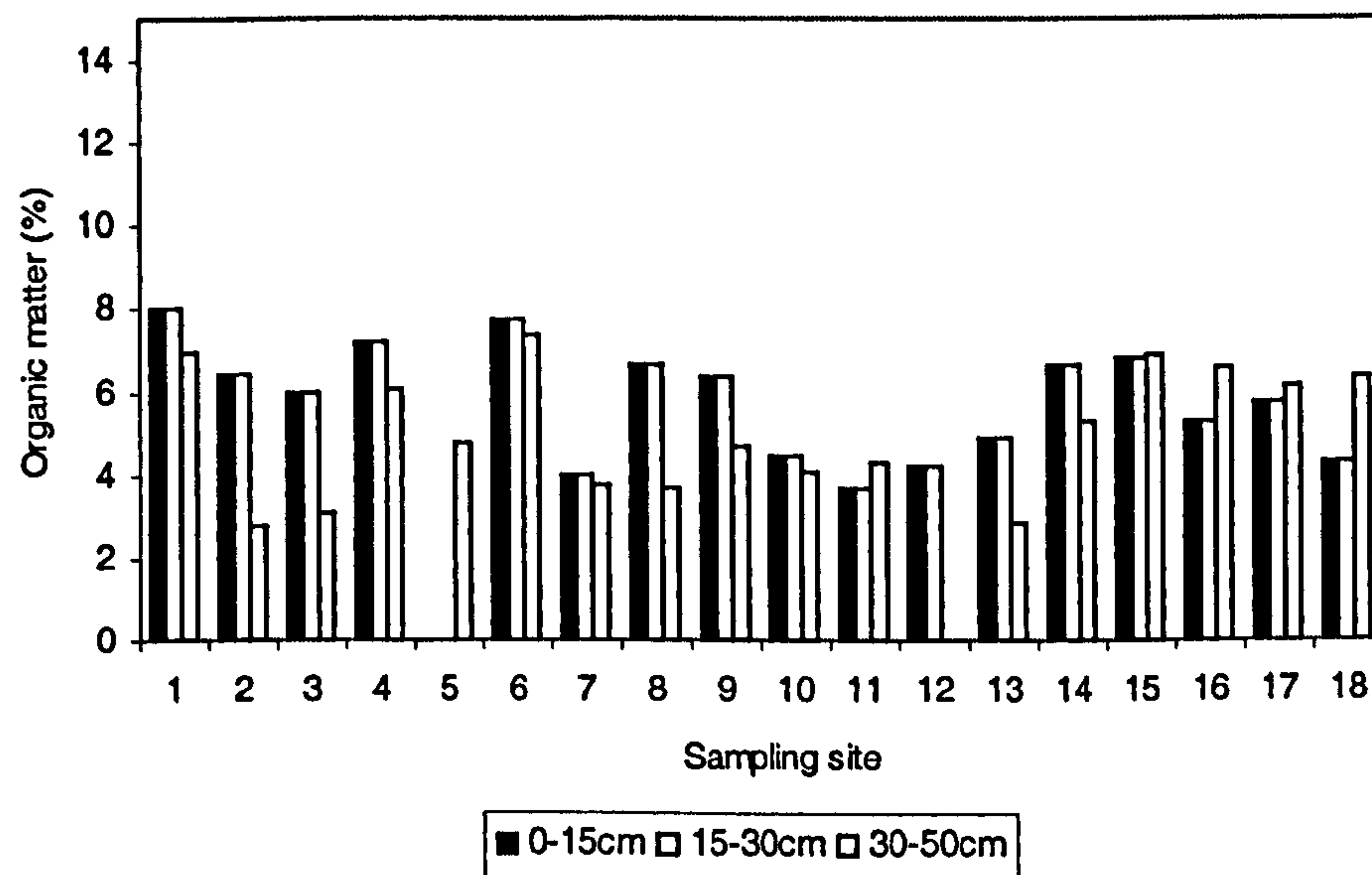


Figure 4.13: Percentage of organic matter in soils from 3 different depths of 18 sampling points.

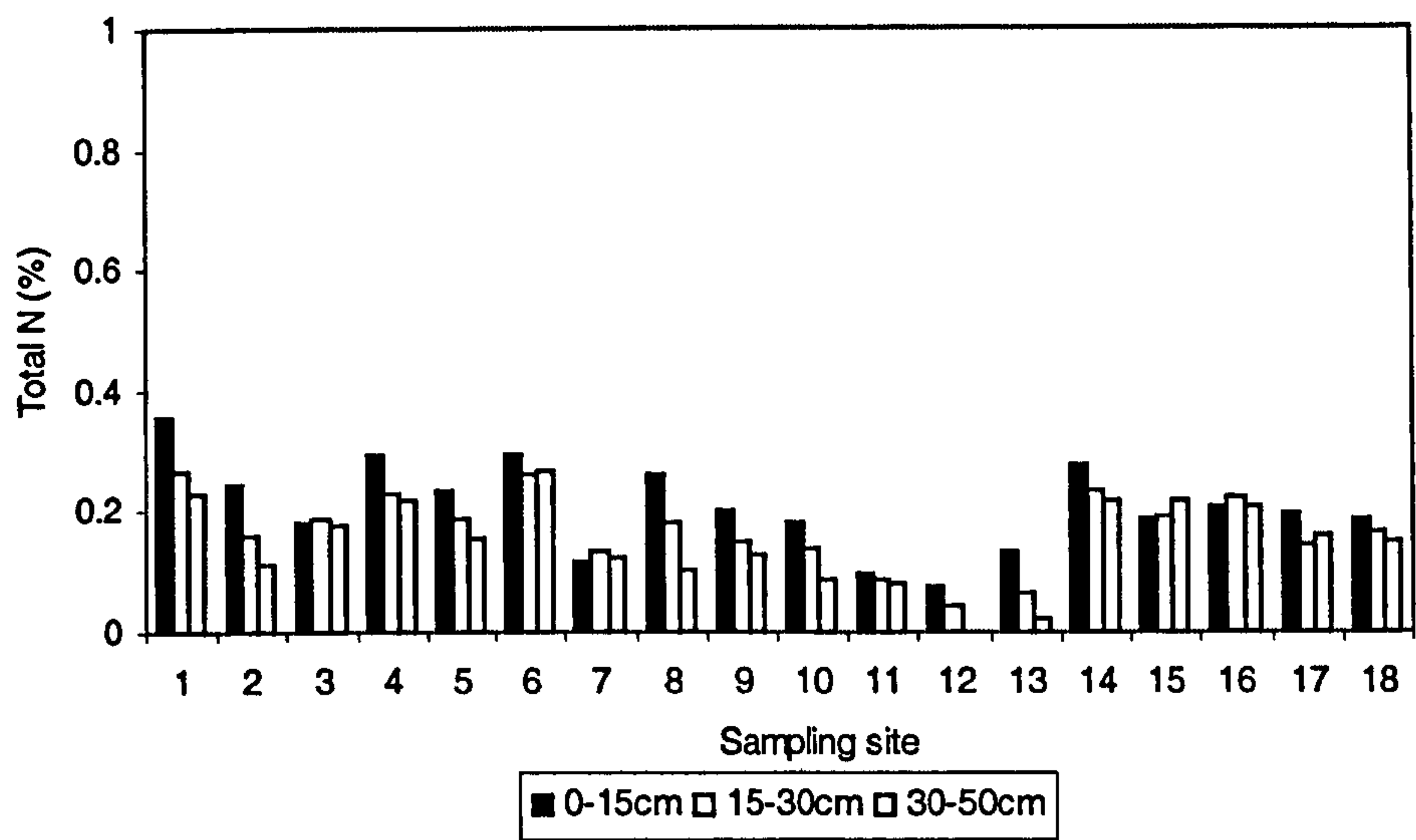


Figure 4.14: Percentage of the total-N in soils from the 3 different depths of 18 sampling points.

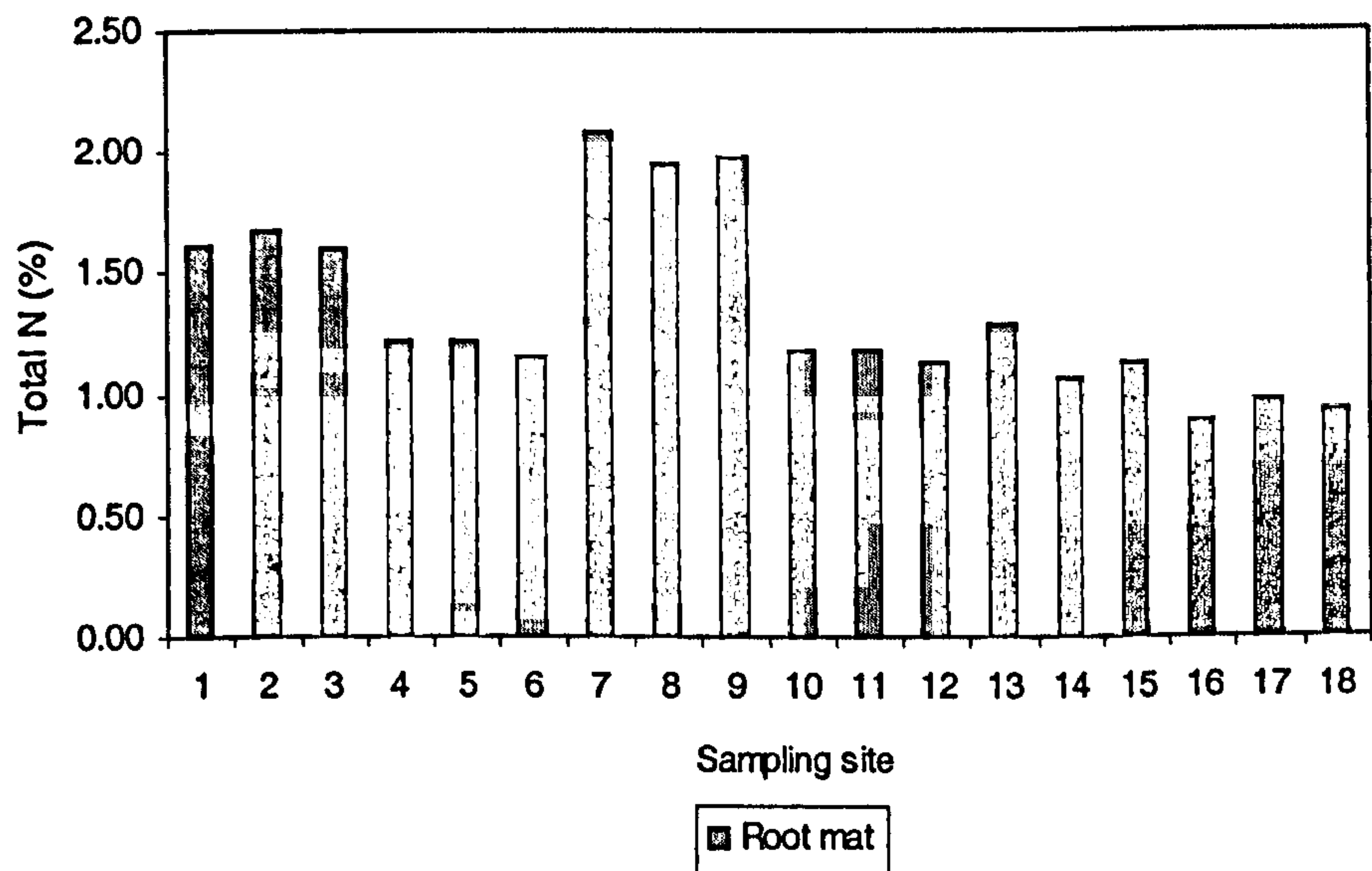


Figure 4.15: Percentage of the total-N in root mat from the 18 sampling points.



Figures 4.14 and 4.15 give the total-N in soils and root mat. Total-N and organic matter in the soils are low compared with 0.5 –1% nitrogen and 10% organic matter in Scottish soils and variable between 0.08% in surface layers of the soil 13 and 0.36% in soil 1. There is also a lack of general change of soil total nitrogen with depth.

There is a highly significant correlation ( $r = 0.812$ ) between the soil organic matter and total nitrogen, which is shown in Figure 4.16. The root mat contains more nitrogen than the soil samples and they build up slowly by time.

The total-P is shown in Figure 4.17. It shows variable values, which range between 0.08% in surface layers of soil 12 and 0.82% in soil 17. The total-P in soils mostly increases with depth up to 1.46% in lower layer of soil 3. The values are generally higher than the normal soils which is typically 0.07% in U.K's soils (Wild, 1988), except for the soil samples from the east corner of the bottom flat area which have low phosphorus content. The total-P in root mat is nearly similar to the top layer of soils.

The results of total-K are given in Figure 4.18. The total-K is low compared with normal soils which is between 0.2-4% in England (Wild, 1988), and is ranging between 0.06% in topsoil of samples from site 7 to 0.23% in soil 17. The total-K in the root mat is much higher than the soils.

The extractable nutrients in the soils are in the Table 4.1(Valad, 2000), and Figure 4.19, 4.20 and 4.21. The values of the ammonium-N (Table 4.1) are low and variable ranging from 1.3mg/kg in topsoil samples 9 and 15 to 5.2% in soil 13. The ammonium-N is higher in surface layers compared with lower layers. There is no detectable nitrite-N in the soil samples where in only a few soil samples nitrate-N was found in sites 1, 4, 16 and 18.

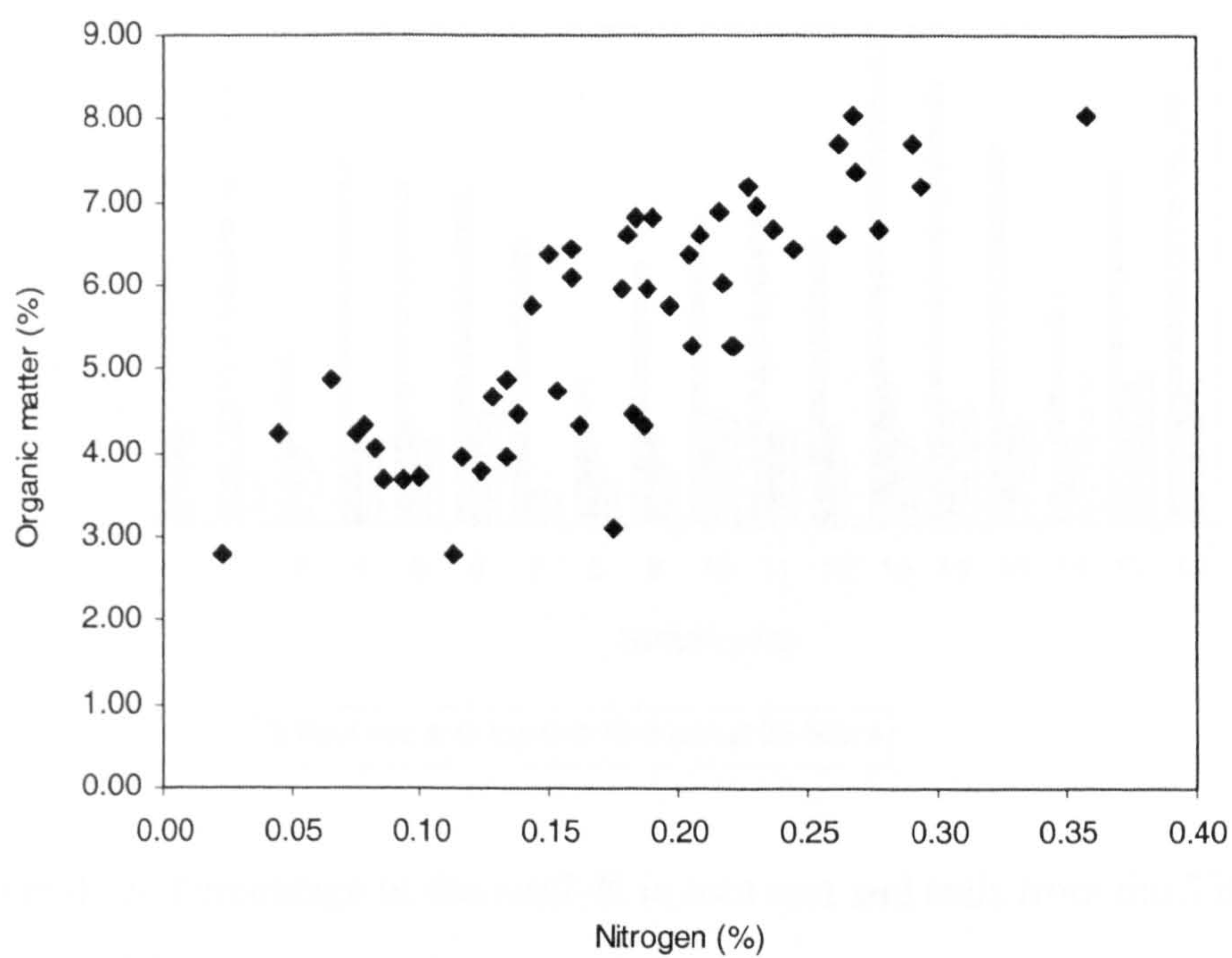


Figure 4.16: Correlation between the percentages of the organic matter and nitrogen in the soils.

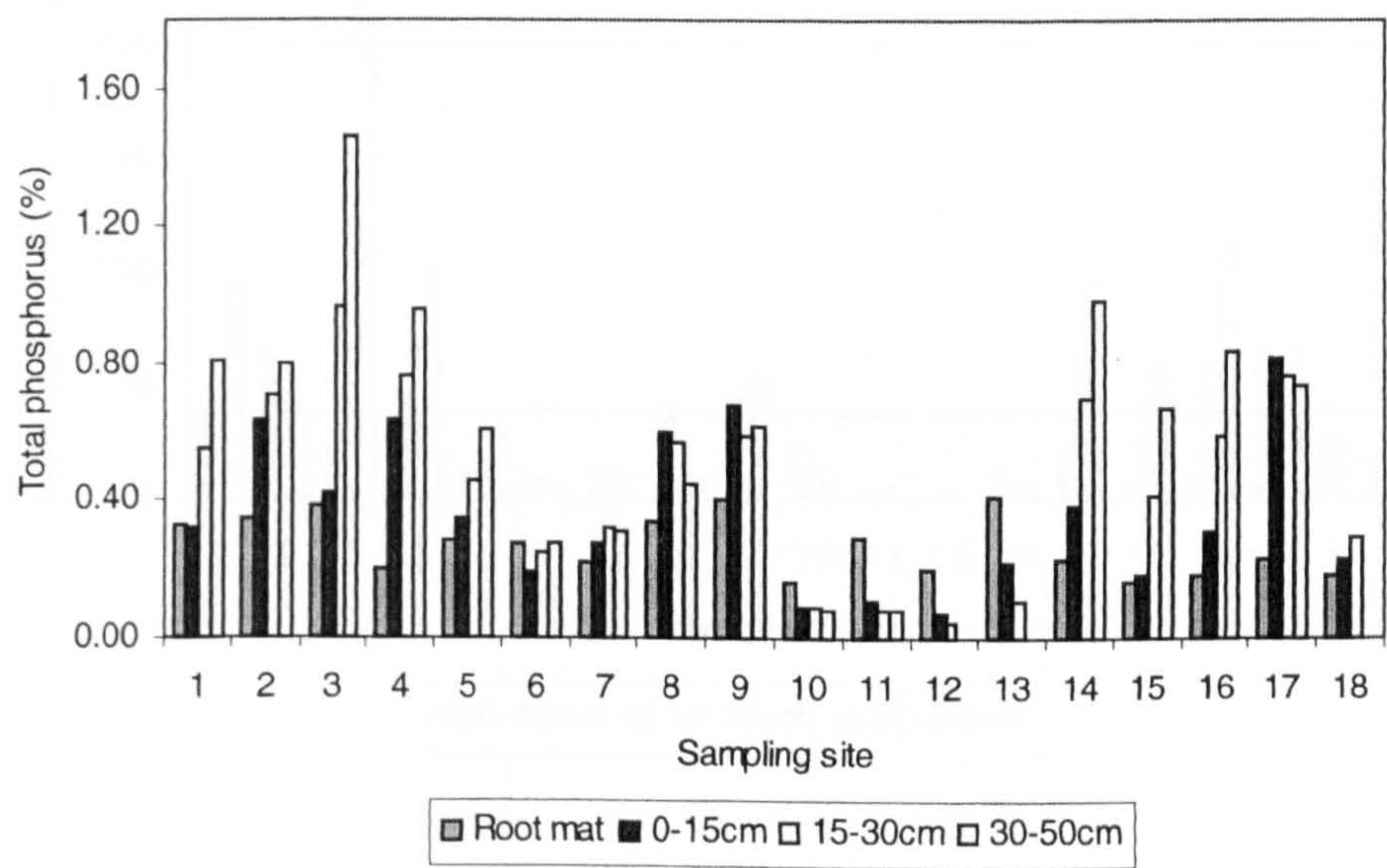


Figure 4.17: Percentage total-P in root mat and soils from the 3 different depths of 18 sampling points.



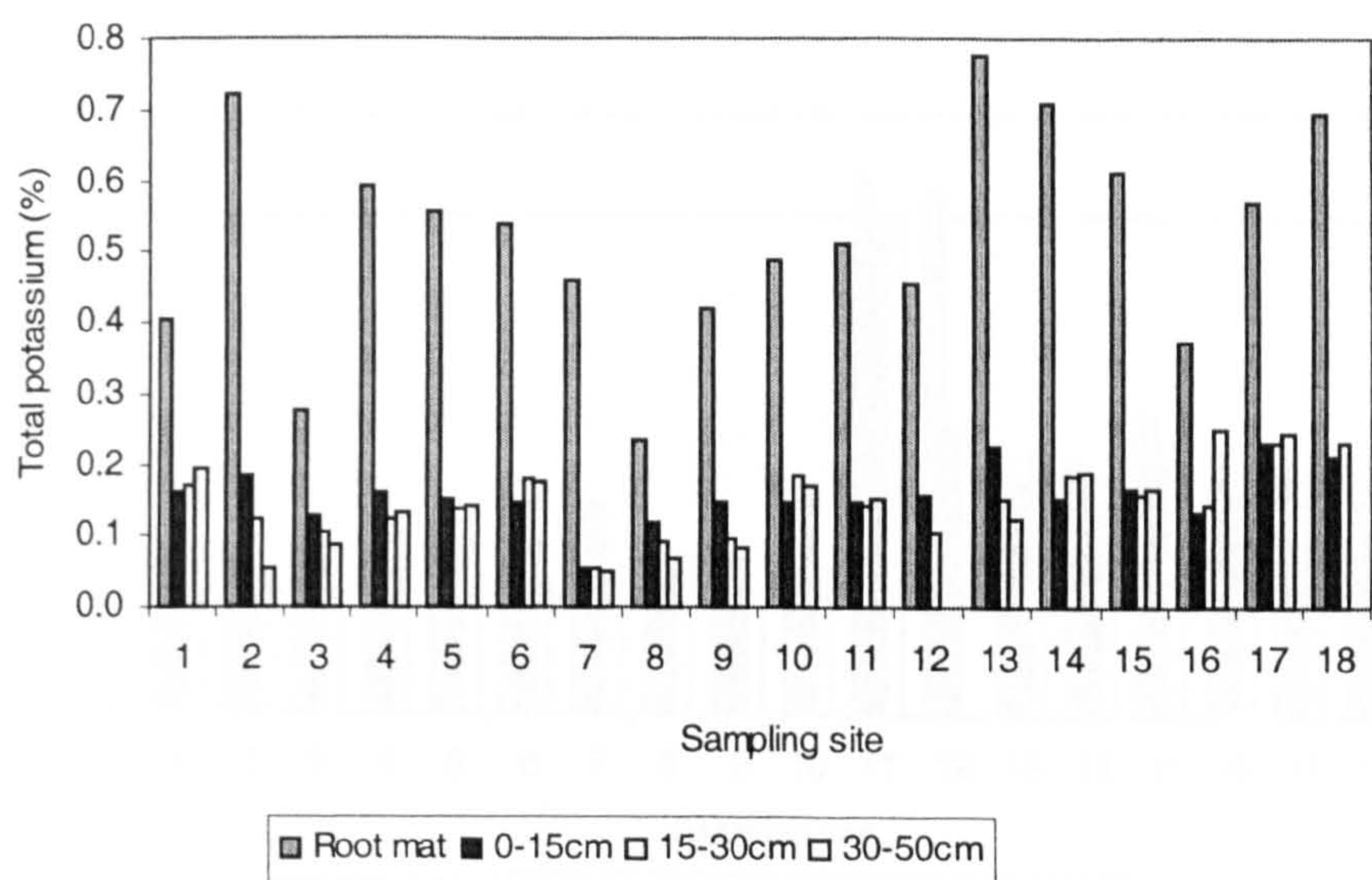


Figure 4.18: Percentage of the total-K in root mat and soils from the 3 different depths of 18 sampling points.

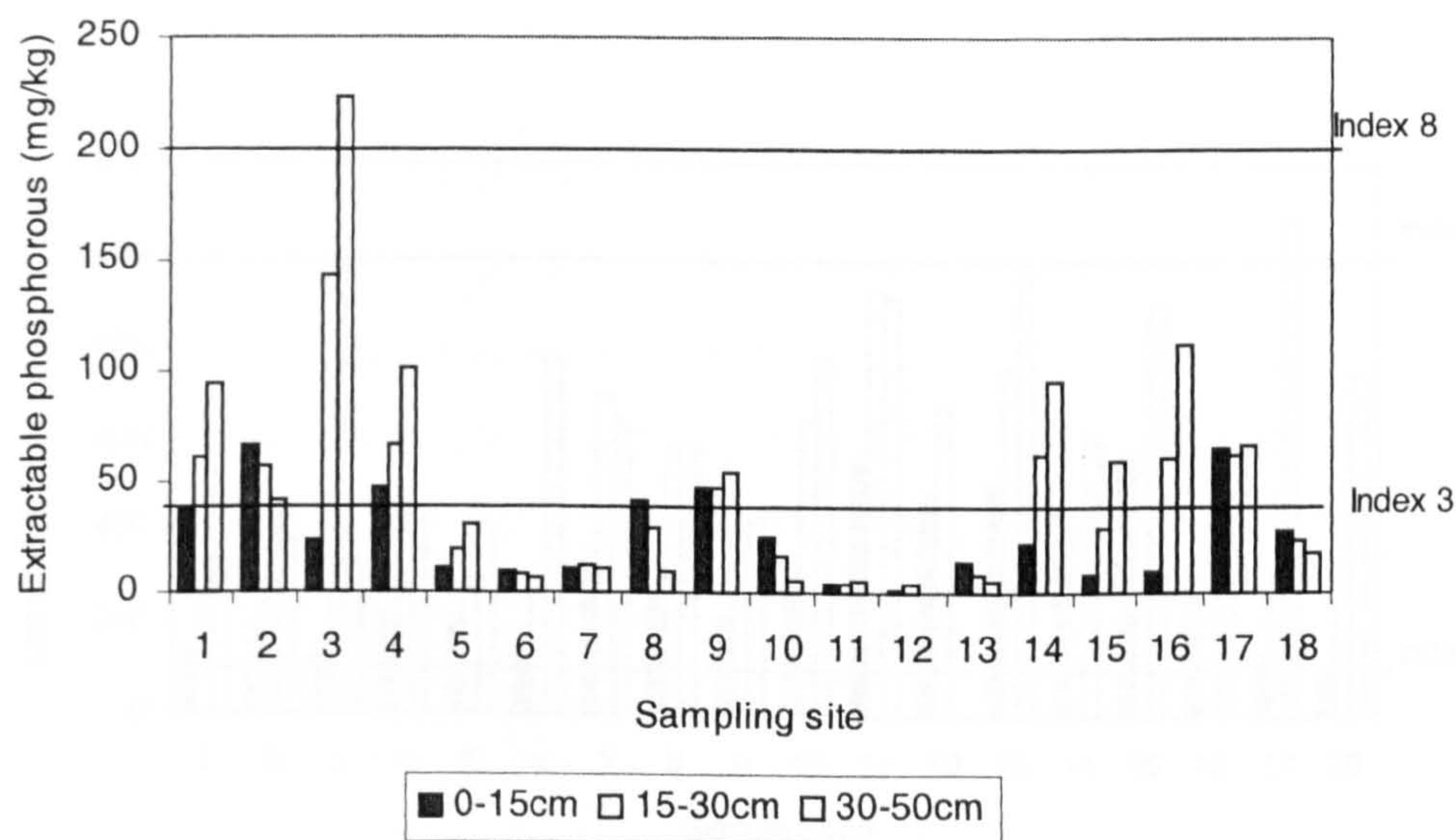


Figure 4.19: Extractable-P in soils from the 3 different depths of the 18 sampling points (mg/kg).



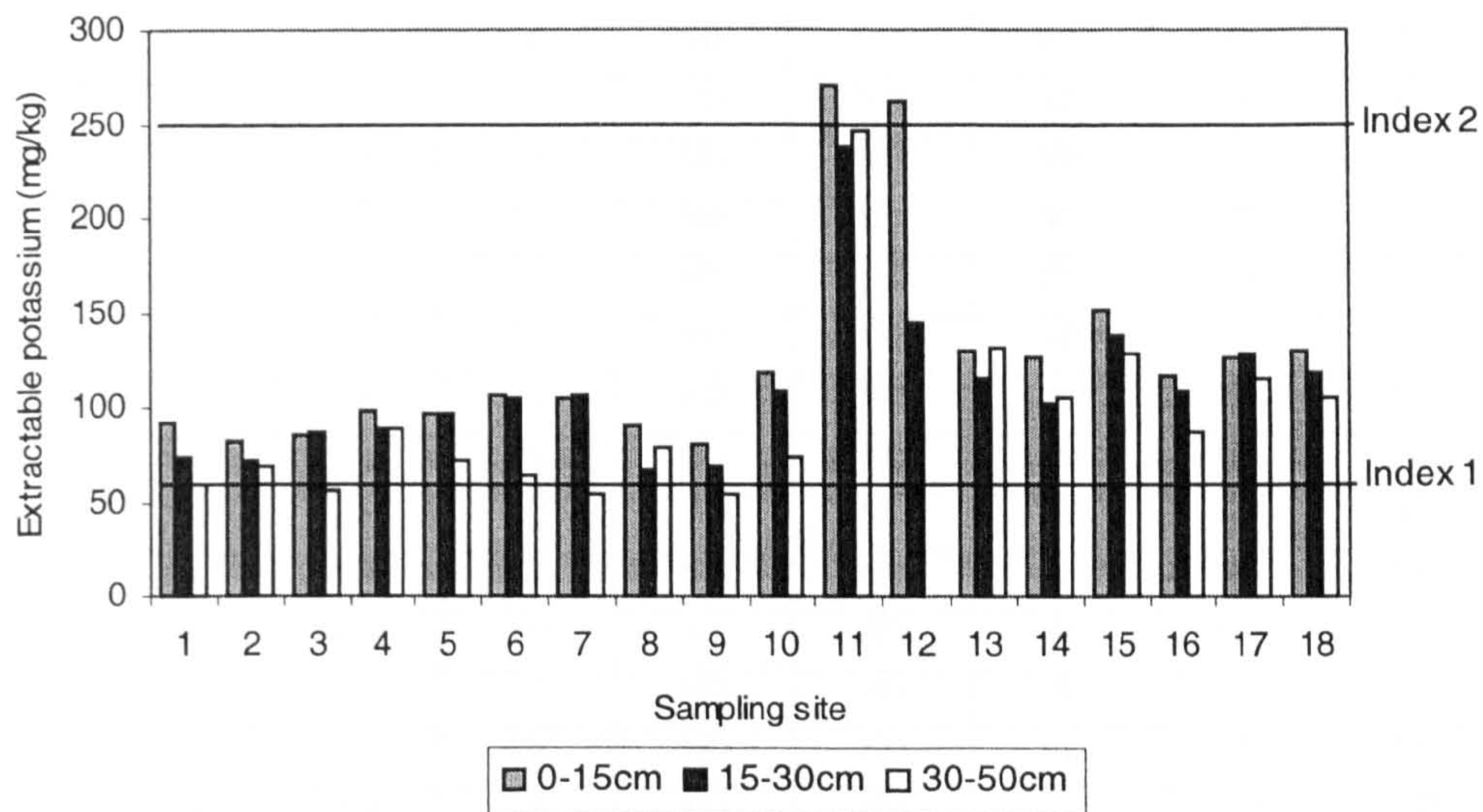


Figure 4.20: Extractable-K in soils from the 3 different depths of the 18 sampling points (mg/kg).

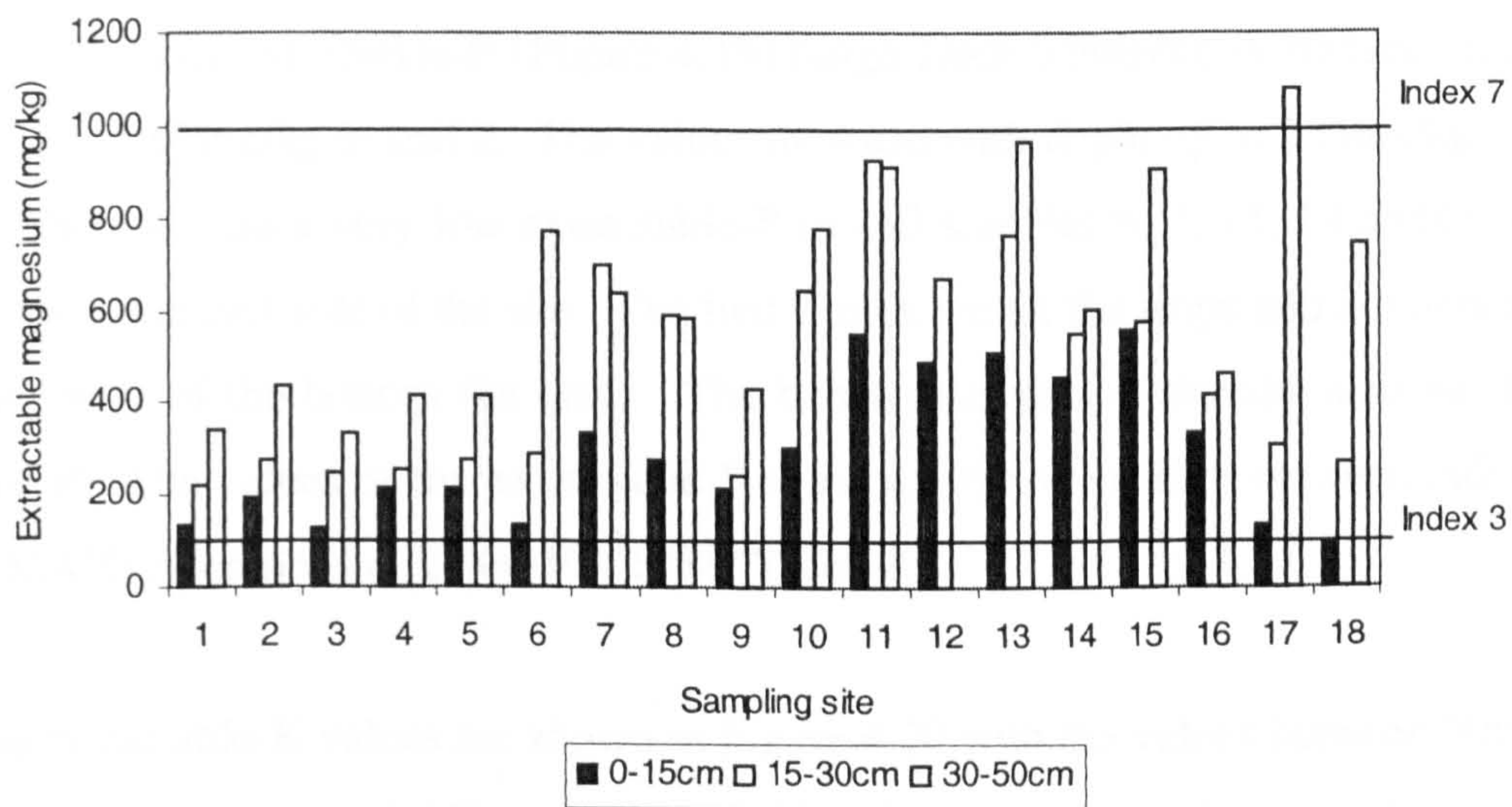


Figure 4.21: Extractable-Mg in soils from the 3 different depths of the 18 sampling points (mg/kg).



Site No	NH <sub>4</sub> <sup>+</sup> -N			NO <sub>2</sub> <sup>-</sup> -N			NO <sub>3</sub> <sup>-</sup> -N		
	0-15cm	15-30cm	30-50cm	0-15cm	15-30cm	30-50cm	0-15cm	15-30cm	30-50cm
1	3.5	1.7	0.8	ND Ψ	ND	ND	2.17	ND	ND
2	2.5	1.0	0.6	ND	ND	ND	ND	ND	ND
3	2.2	0.9	1.0	ND	ND	ND	ND	ND	ND
4	2.1	1.8	0.8	ND	ND	ND	1.62	ND	ND
5	3.5	1.4	2.2	ND	ND	ND	ND	ND	ND
6	2.4	1.5	1.1	ND	ND	ND	ND	ND	ND
7	2.5	0.5	1.4	ND	ND	ND	ND	ND	ND
8	3.6	1.4	1.3	ND	ND	ND	ND	ND	ND
9	1.3	1.5	0.9	ND	ND	ND	ND	ND	ND
10	4.4	1.8	0.8	ND	ND	ND	ND	ND	ND
11	1.5	0.8	0.5	ND	ND	ND	ND	ND	ND
12	1.4	1.3	m*	ND	ND	m	ND	ND	m
13	5.2	0.6	1.0	ND	ND	ND	ND	ND	ND
14	3.0	1.4	1.3	ND	ND	ND	ND	ND	ND
15	1.3	0.5	0.8	ND	ND	ND	ND	ND	ND
16	4.4	1.4	1.6	ND	ND	ND	ND	ND	2.51
17	2.4	0.9	0.9	ND	ND	ND	ND	ND	ND
18	1.4	1.5	1.0	ND	ND	ND	1.42	1.89	11.5

Table 4.1: Concentration of extractable inorganic nitrogen (mg/kg) in soils.

\* m: are missing samples and Ψ ND: Non detectable < 0.005 mg/l extract solution or 0.1 mg/kg soil.

The values of extractable-P (Figure 4.19) range from 0.9mg/kg in surface layers of soil 12 to 67mg/kg in soil 2. The values increase with depth up to 223mg/kg in soil 3. There is also a very low extractable-P in soil samples 6, 7, 11, 12 and 13 which are from the east side of the site. The first 2 sites are on the slope and 3 others are in the corner of the bottom flat area. The bottom flat part of the site also has a low total-P. The values of the extractable-P show a great variability between index 0-8 in MAFF reference book (MAFF, 1988) Table 4.2.

The extractable-K values are shown in Figure 4.20 with the values between 50mg/kg in topsoil of site 9 and 150mg/kg in soil 15. The values are almost uniform across the site with the exception of high values in soils 11 and 12. The extractable-K values are mainly between the index 1 and 2 in the MAFF reference book (MAFF, 1988).



The results for the extractable-Mg (Figure 4.21) show high values, which increase with depth. They are between index 3 –7 of the MAFF reference book. These values may be related to high pH and some impurity of carbonate used in the industrial work.

The ICRCL (Interdepartmental Committee on the Redevelopment of Contaminated Land) book was used as a reference for comparing the content of the Chromium and the other metals in order to find out their presence and/or degree of the contamination.

The values are as follow:

Contaminant	Planned uses	Threshold (mg/kg)
Chromium(VI)	Domestic garden, allotments	25
	Parks, playing field, open space	
Chromium(total)	Domestic garden, allotments	600
	Parks, playing field, open space	1000
Lead	Domestic garden, allotments	500
	Parks, playing field, open space	2000
Copper	Any uses where plants are to be grown	130
Nickel	Any uses where plants are to be grown	70
Cadmium	Domestic garden, allotments	3
	Parks, playing field, open space	15
Zinc	Any uses where plants are to be grown	300

The total amount of the metals in the soils and root mat are given in the Figures 4.22, 4.23, 4.24, 4.25 and 4.26. Most of the values for total-Cr (Figure 4.22) are more than 1000mg/kg chromium (the threshold value) in the soil. The higher values are from the sampling points from the east side of the slope area and the highest



value of 15000mg/kg, which is from sampling point 11 at the bottom part of the flat area. Comparing the values of the soils total chromium with the ICRCL book shows these soils are highly contaminated with the chromium and must be regarded as a hazardous site from the chromium point of view, both for the open space and the domestic gardens. Water with a high level of chromium and yellow colour can also leach from the site especially from the slope and increase the risk of the exposure of the chromium to the children and adults.

The total-Cu values (Figure 4.23) are between 51–145mg/kg in topsoil of sampling points 18 and 1 respectively. The values are mainly less than the ICRCL threshold value of 130mg/kg copper in the soil, except for the site number 4, which is 222mg/kg in lowest depth.

The values for the total-Ni (Figure 4.24) are between 80-550mg/kg in topsoil of sampling points 18 and 16 respectively. The content of this metal is high compared with the ICRCL book, which is 70mg/kg nickel in the soil.

The total lead (Pb) in these soil samples (Figure 4.25) is from about 110-800mg/kg in the topsoil of sampling points 10 and 1 respectively. The values are less than the ICRCL threshold value which is 2000mg/kg lead in the soil except for the lower depth of the soil number 15 with 2000mg/kg lead content.

Total amounts of zinc in the soils (Figure 4.26) are  $\leq$  300mg/kg of the ICRCL threshold value except for sampling points 2 and 10 with 600 and 1200mg/kg respectively.

Cadmium was not detected in the 3 depths of any soil sample.

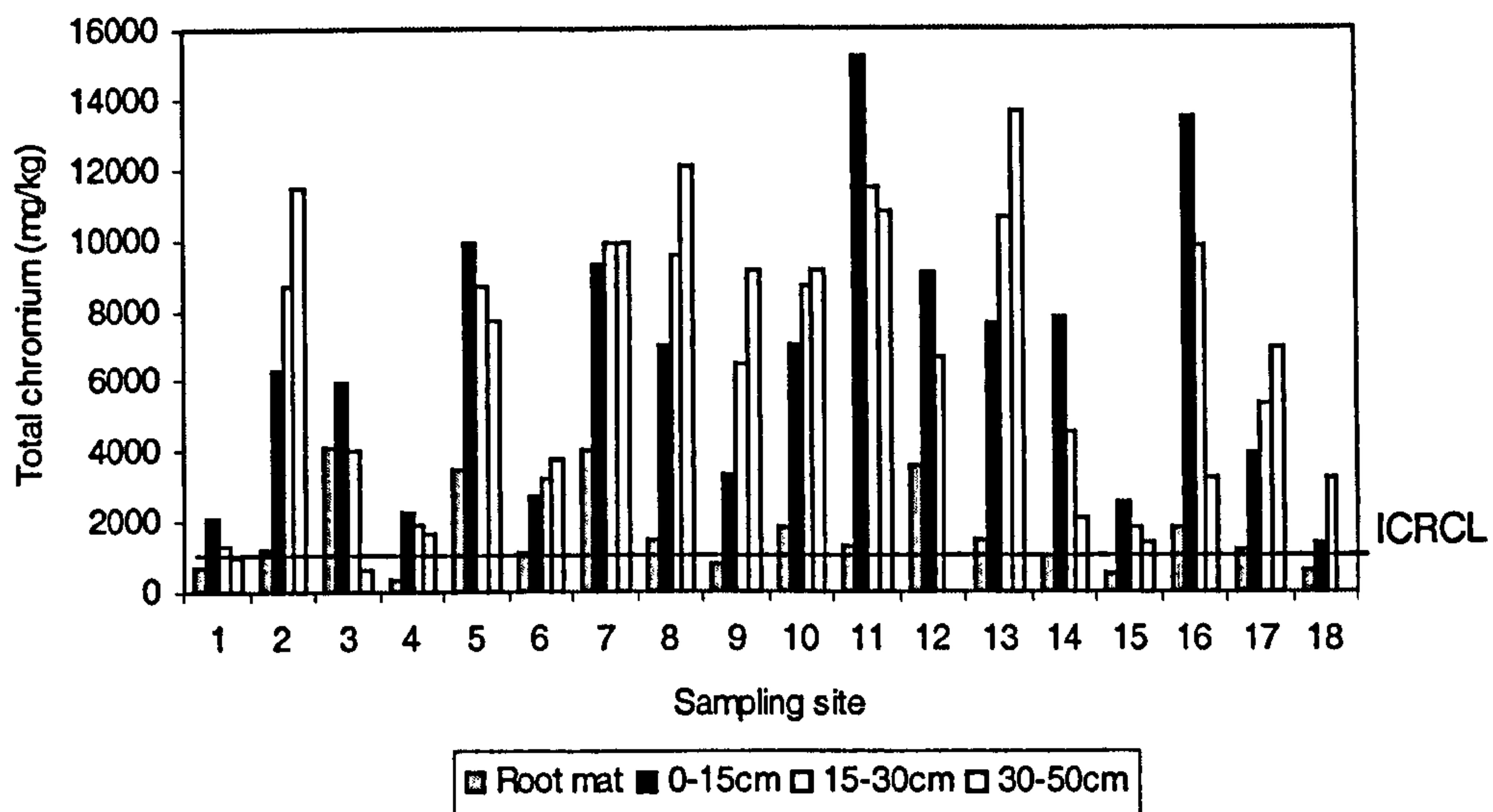


Figure 4.22: Total-Cr in the root mat and soils from the 3 different depths of the 18 sampling points (mg/kg).

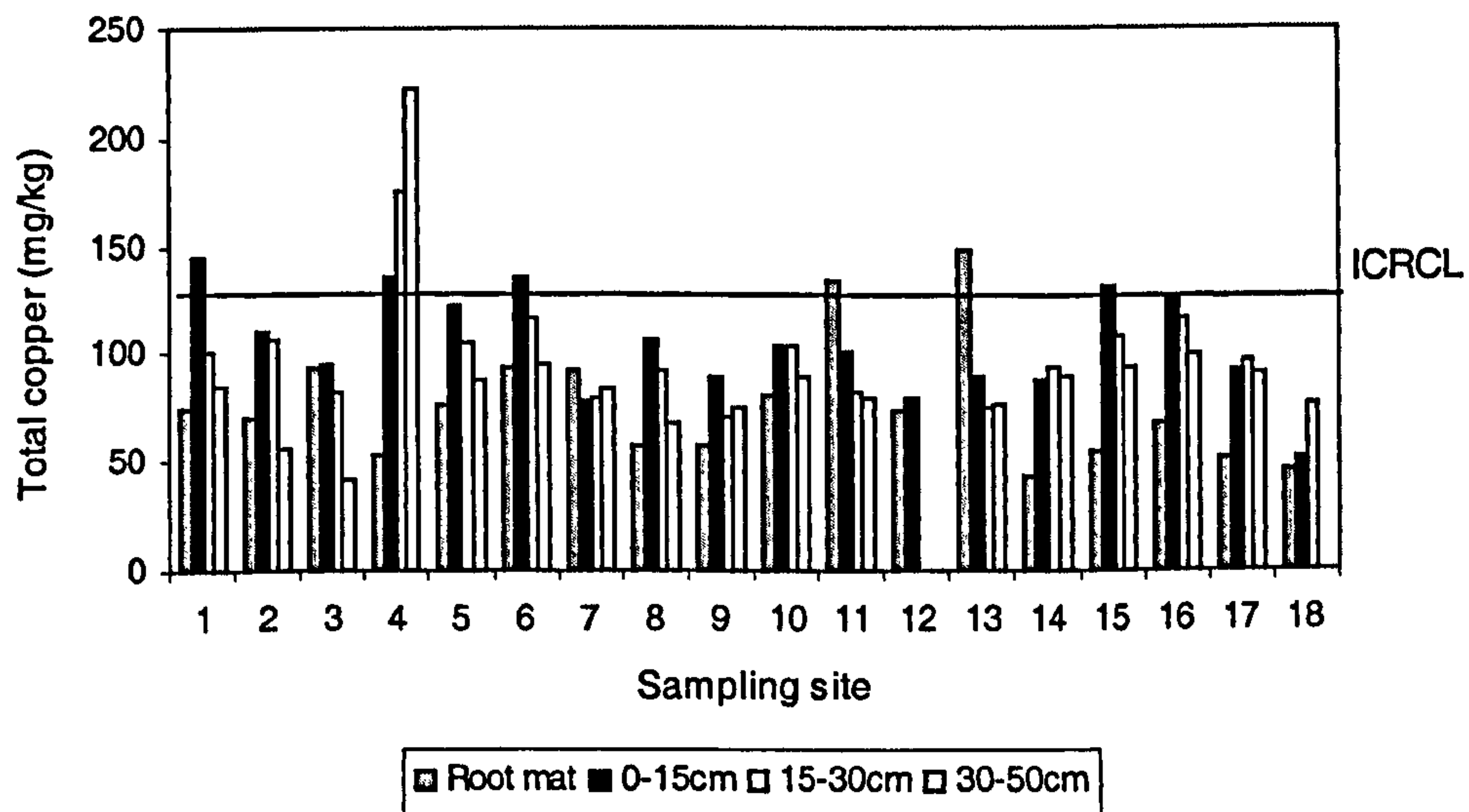


Figure 4.23: Total-Cu in the root mat and soils from the 3 different depths of the 18 sampling points (mg/kg).

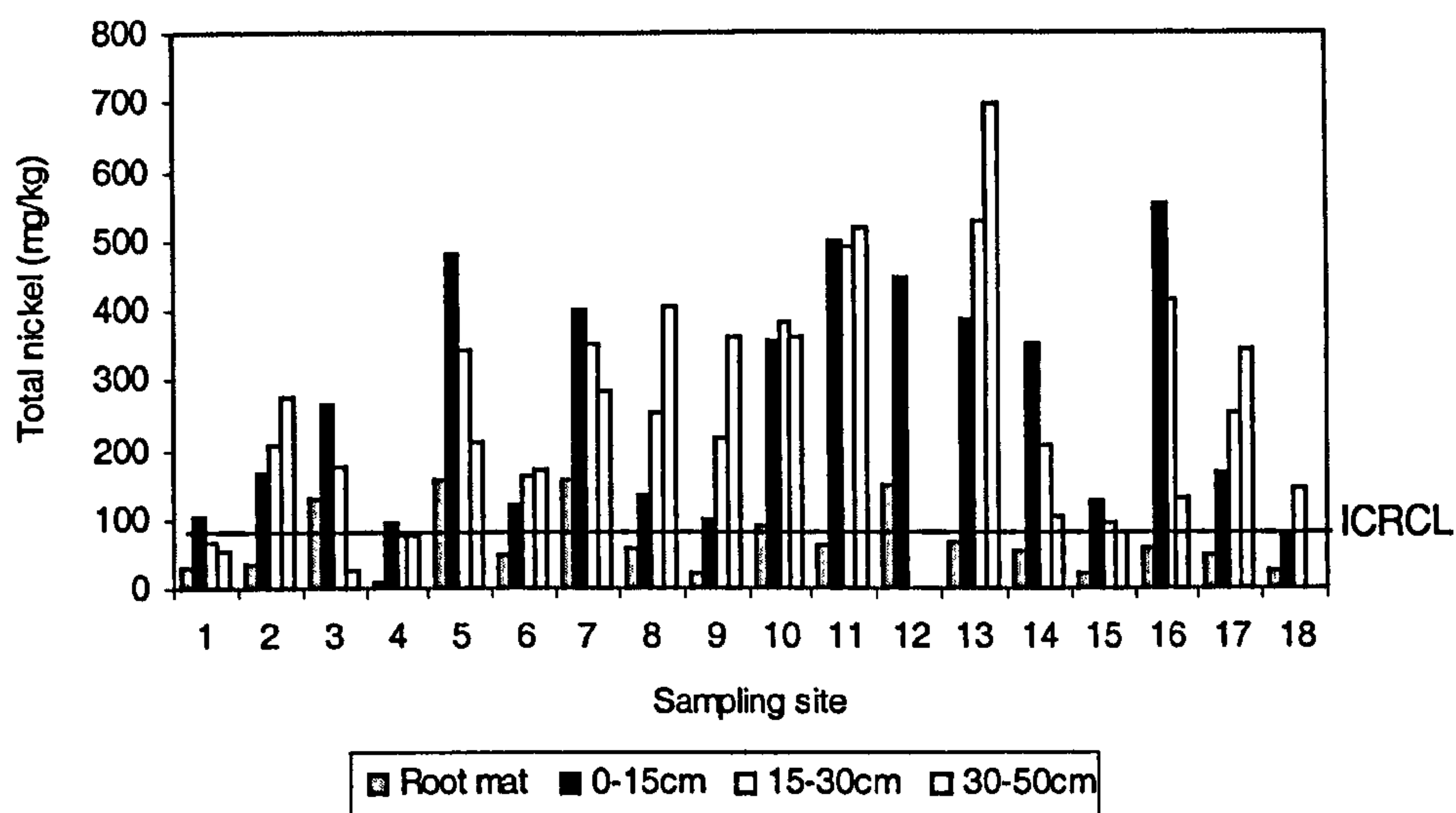


Figure 4.24: Total-Ni in the root mat and soils from the 3 different depths of the 18 sampling points (mg/kg).

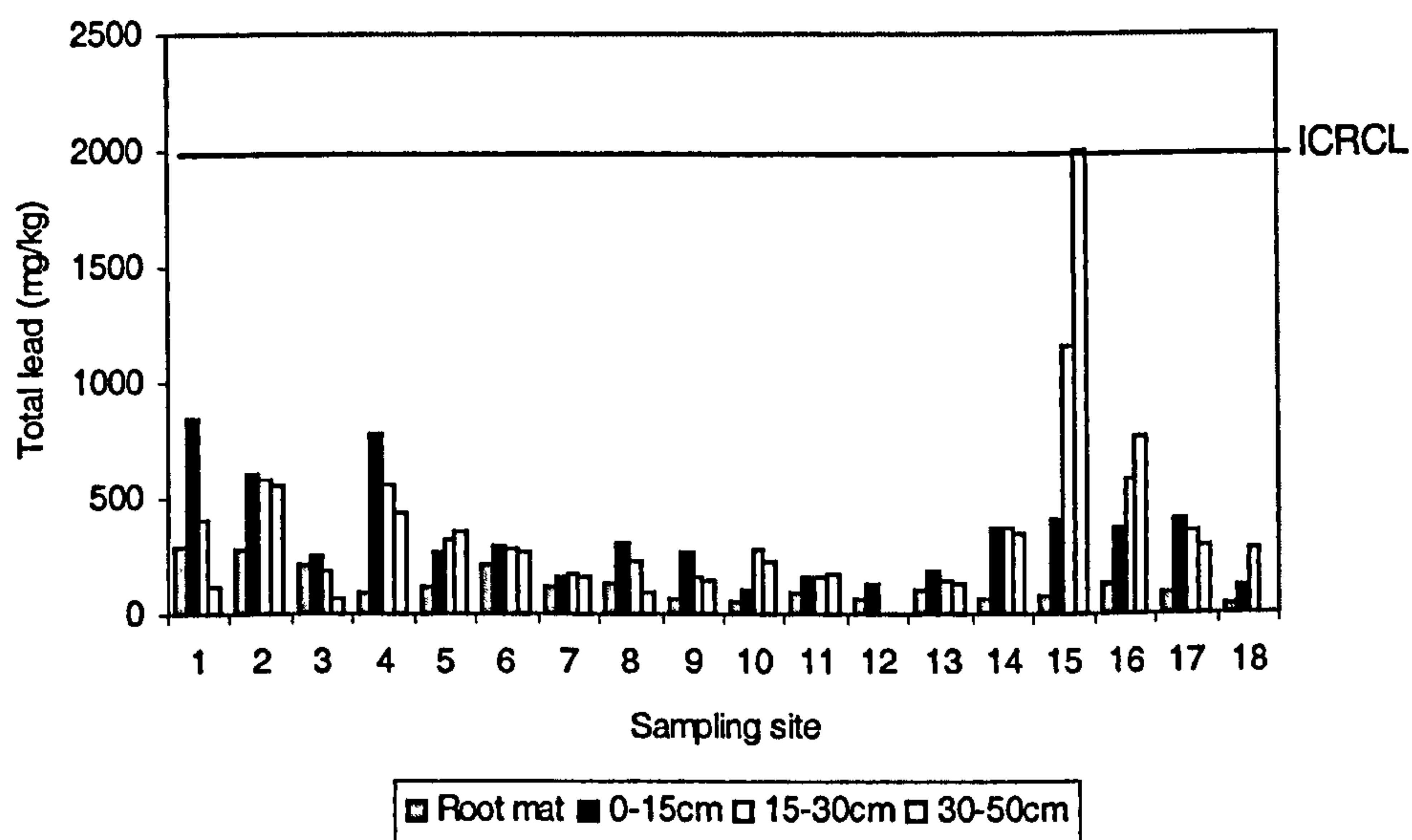


Figure 4.25: Total-Pb in the root mat and soils from the 3 different depths of the 18 sampling points (mg/kg).



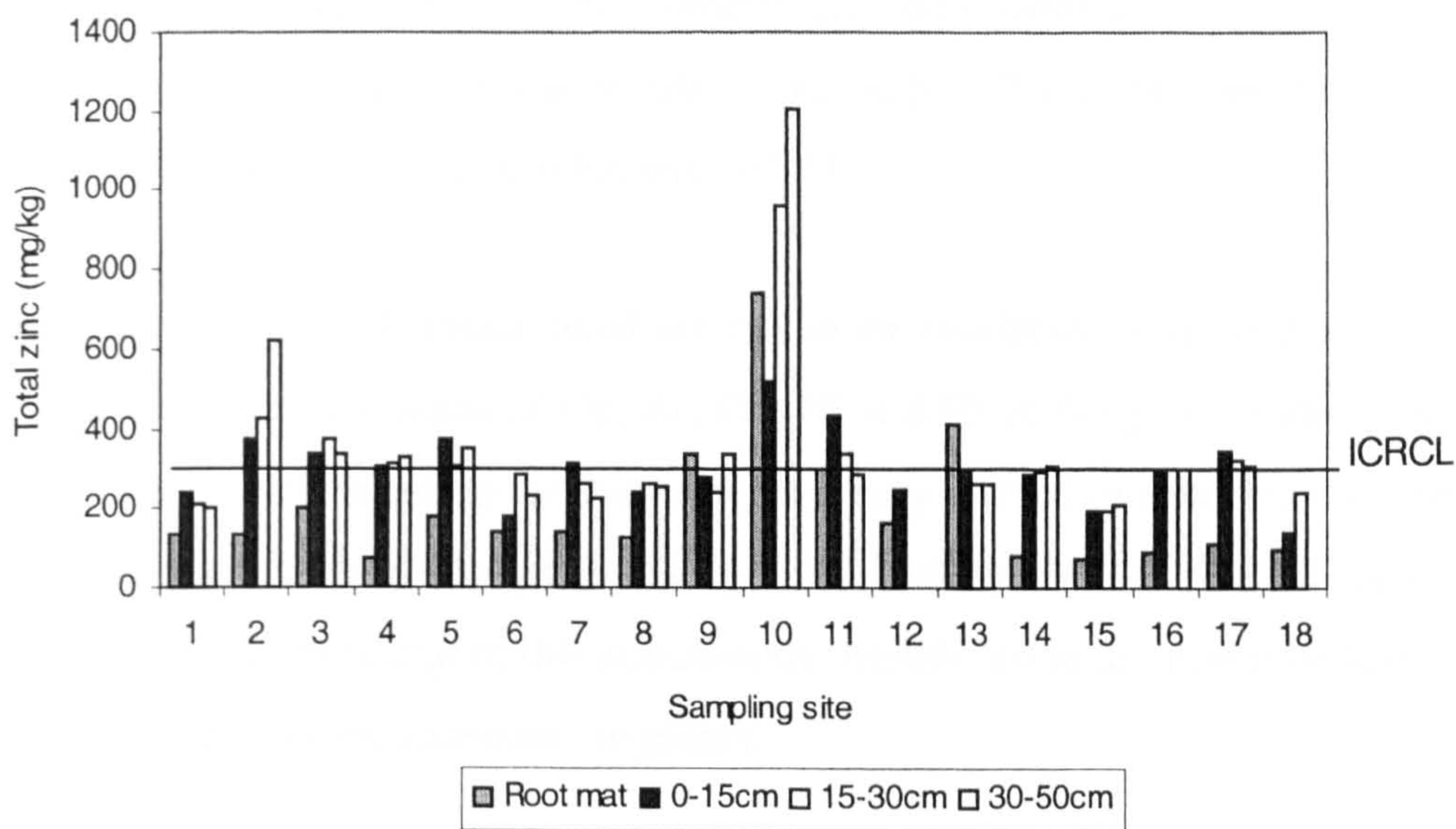


Figure 4.26: Total–Zn in the root mat and soils from the 3 different depths of the 18 sampling points (mg/kg).

Index	Phosphorus (mg/litre)	Potassium (mg/litre)	Magnesium (mg/litre)
0	0-9	0-60	0-25
1	10-15	61-120	26-50
2	16-25	121-240	51-100
3	26-45	241-400	101-175
4	46-70	401-600	176-250
5	71-100	601-900	251-350
6	101-140	901-1500	351-600
7	141-200	1501-2400	601-1000
8	201-280	2401-3600	1001-1500
9	over 280	Over 3600	over 1500

Table 4.2: classification of soil analysis results for available nutrients (MAFF Reference Book 209, 1988).



In general the soils contain variable amounts of metals which are scattered in different parts of the site. The availability of metals like nickel, lead, copper, zinc, and cadmium depend on the total content and their mobility in the soil. Liming reduces the availability of these metals in the soils. That is because lime increases the pH of the soils (Mengel and Kirkby, 1978).

Therefore in higher pH these metal are not in an available form for plants. On the other hand the translocation of Cu, Zn, Cd, Ni and Pb in the plant is slow and those metals which were taken up by roots are not easily moved to fresh leaves especially in annual plant material like grass (Mengel and Kirkby, 1978). The pHs of the soils from this site are high and in this situation the metals are in an immobile form which probably might not be accessible to plants.

The concentrations of chromium, nickel and lead in the root mat are less than the concentration of those metals in the surface soils. The zinc and copper are micronutrients and organic matter of root mat naturally contains some zinc and copper.

Table 4.3 shows the values for the water soluble chromium and the potassium sulphate extractable chromium. Water extractable chromium ranges from 0mg/kg in surface of most of the soils to 821mg/kg at 30-50cm depth of site 8. The potassium sulphate extractable chromium ranges from 5mg/kg in some surface sites to 1553mg/kg at 15-30cm depth of site 7. The samples from the sites 2, 7 and 8 give the highest values of the water and potassium sulphate extractable chromium. These samples have carbonate waste material near the surface of the soil profile. There is also a high extractable-Cr from 0-15cm depth of site 7 compared with the similar depth of the other soil samples. The sites 7 and 8 are on the slope area at the east side. Most soil samples from the depth 0-15cm have the lowest values of extractable chromium compared with the other depths.



Site NO	mg/l Water Extractable Cr (Dionex)			mg/l K <sub>2</sub> SO <sub>4</sub> Extractable Cr. (A.A)		
	0-15 cm	15-30 cm	30-50 cm	0-15 cm	15-30 cm	30-50 cm
1	0	2.6	3.6	10.5	9.1	9
2	2.22	7.8	138	13.5	107	207
3	0	2.7	2.4	6.1	8.7	11
4	0	1.4	3.5	5	7.6	12.2
5	0	3.4	29.8	6	23.7	46.3
6	0	0	0	4.5	4.1	5
7	40.8	567	387	62.2	1553	888
8	0	13.1	821	5.3	35	1438
9	0	0.9	7	5.6	14.6	28
10	0	4.1	1.6	4.8	6.8	9.1
11	0.7	1.7	1.7	4.8	5.7	7
12	0.7	1	m*	5.3	m	m
13	0	3.1	3.4	4.7	7.2	9
14	0	1.4	12.3	4.8	5.4	6.8
15	0.83	0	2.3	5	5	7.9
16	0	3.9	11.7	5.6	14.1	25.1
17	1.2	1.5	8.5	7.1	9.9	1.2
18	0	0	2.9	3.5	5.5	5.6

Table 4.3: Extractable chromium with water and K<sub>2</sub>SO<sub>4</sub> from different depths (mg/l).  
 \*: are the missing values

Comparing the water extractable-Cr and the potassium sulphate extractable chromium of the soils shows a higher amount of chromium is extracted with a salt solution extractant from the soil. The higher amount of the potassium sulphate extractable chromium of the soils is probably because the anion and cation of the salt solution could release more Cr(III) or Cr(VI) from the exchange sites of the soils. The water extractable-Cr was measured with ion exchange chromatography which determines only the anion that is the Cr(VI), but the potassium sulphate extractable chromium was measured with the Atomic Absorption that measured any chromium in the solution including the Cr(III) and Cr(VI).

Comparing the total chromium with the water soluble chromium of the soils shows that only a small portion of the chromium content of these soils is soluble. This shows that probably the majority of the total chromium in these soils is in the form of Cr(III). The solubility of the Cr(III) depends on the pH of the soil and is lower in higher pH. Therefore it can be assumed that the soluble Cr(III) in these soils is also low.



The NPK and the metals in the plant material are given in the Table 4.4. Despite the presence of the copper, lead, chromium and nickel in the root mat there is no detectable content of these metals analyzed especially chromium in the plant digest solution except for zinc. As it was mentioned earlier zinc is a micronutrient and can be found in leaves naturally. The root mat is not renewed each year therefore it can accumulate the chromium by time, but the annual growth like the grass is renewed every year, and accumulation of the chromium is low or not detectable.

Concentration of total nutrients and six metals in plants									
Site NO	% N	% P	% K	mg/kg Cr	mg/kg Pb	mg/kg Cu	mg/kg Cd	mg/kg Ni	mg/kg Zn
1	1.35	0.33	2.33	NDψ	ND	ND	ND	ND	40
2	1.46	0.33	2.35	ND	ND	ND	ND	ND	30
3	0.90	0.31	2.14	ND	ND	ND	ND	ND	42
4	1.03	0.28	2.60	ND	ND	ND	ND	ND	43
5	0.95	0.27	2.08	ND	ND	ND	ND	ND	32
6	1.11	0.26	1.98	ND	ND	ND	ND	ND	43
7	0.79	0.22	0.36	ND	ND	ND	ND	ND	26
8	1.29	0.34	0.47	ND	ND	ND	ND	ND	30
9	1.62	0.41	0.50	ND	ND	ND	ND	ND	37
10	0.86	0.21	1.79	ND	ND	ND	ND	ND	29
11	0.74	0.21	1.93	ND	ND	ND	ND	ND	22
12	1.06	0.20	1.68	ND	ND	ND	ND	ND	47
13	1.03	0.29	2.22	ND	ND	ND	ND	ND	33
14	1.31	0.27	1.75	ND	ND	ND	ND	ND	37
15	0.76	0.27	2.05	ND	ND	ND	ND	ND	29
16	0.90	0.23	1.44	ND	ND	ND	ND	ND	34
17	0.94	0.25	1.54	ND	ND	ND	ND	ND	31
18	0.75	0.22	1.53	ND	ND	ND	ND	ND	31

Table 4.4: Total of nutrient and six different metals in the grass.

ψ: ND values are values under the certain limit, which could be detected for each certain metal. These limits are 0.02 mg/l for Cu and Cr, 0.005 mg/l for Zn, 0.1 mg/l for Pb, 0.02 mg/l for Ni and 0.01mg/l for Cd in digest solution. These values corresponds to 5mg/kg for Cu and Cr, 1mg/kg for Zn, 25mg/kg for Pb, 5mg/kg for Ni and 2.5 mg/kg for Cd in plant materials.

Some investigators did not observe the chromium in the plant (Cary and Kubota, 1990). They studied the effect of chromium concentration of soil on the chromium concentration in plants and measured the Cr and Ti in soil and calculated the ratio of

Cr to Ti in soil. They also measured the titanium (Ti) and chromium (Cr) in the plants and corrected the value of the chromium, assuming that titanium is a measure of the soil in a plant tissue. They considered that the chromium measured in plant material is because the soil particles may become lodged in the plant parts as they come up from the soil and in case of leaves the most likely contamination is the fine dust. They expressed their doubt about any chromium absorbed to the plant from the soils. On the other hand some other investigator found chromium in plant material. McGrath (1982) found the chromium was absorbed by the oat both in the Cr(III) and the Cr(VI) forms when he added 750mg/kg Cr to the nutrient solution and the soil. He used acidic soil and alkaline soil and added similar chromium to them and found more chromium was present in acid soil solution than the alkaline soil.

Yield and nutrients in the soil may vary during different times due to temperature, rainfall or plant uptake (Baggs et al., 2000).

Six sampling points were chosen from the whole site based on the three parts of the site i.e. flat part on the top of the slope, the slope and the flat part at the bottom of the slope (Figure 4.11). Sampling point 1 was selected from the flat part of the top of the site, which had a good grass cover and some small birch trees towards the back and close to the railway embankment. The profiles had loose ash material. Two sampling points (2 and 3) were chosen on the slope area. Sampling point 2 was from the planted area west half of the slope. This area had a dense stand of small birch trees with some grass and a loose ash profile with many tree roots. Sampling point 3 was located on the unplanted east side of the pathway on the slope area. This was very poorly vegetated with sparse birch trees. The profile contained loose ash with the calcium carbonate waste material at less than 30cm depth. Three soil samples 4 to 6 were chosen from the flat part of the site at the bottom of the spoil, which had patchy grass. The profile had uniform ash material with the very compact material below the 15cm depth. Sampling point 4 was right at the bottom of the east side of the slope area and quite often was very wet. Sampling point 5 was at the center of the east side of the flat area and sampling point 6 was at the center of the west side of the flat area.



The results are presented in Figure 4.27 to 4.37. The grass yield obtained is different at different times and also at the different sampling points (Figure 4.27). It is low in winter time and high in summer time. Various factors are required for plant growth such as light, CO<sub>2</sub>, temperature, mineral nutrition and water. The total dry matter production of plants is first directly related to photosynthesis, the primary process of synthesis of organic compounds, which is conversion of light energy to chemical energy. Therefore plants grow better in summer, due to higher temperatures and photosynthesis while in winter low temperature causes slower plant growth. The low temperatures in winter also cause low activity and growth for the soil bacteria. In the growing season they are activated and provide better situation for growing plants (Tate, 1995). As it can be noticed soil sample from sampling point 3 has the lowest yield through the all seasons.

Figures 4.28, 4.29 and 4.30 show the amount of total yields of N, P and K in one square meter plants at different times. In all three figures the amount of nutrients are low in winter time and high in growing season which is mainly due to the better growth of the plants in summer time and lower growth in winter time. The amount of nutrients in square meter obtained by multiplying of dry matter yield in square meter to percent of each nutrient (N, P and K) in plant. The figures also show the lower nutrient content of plants growing on sampling point 3 compared with the other sampling points.

The extractable NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N and total-N from different times are given in Figures 4.31, 4.32 and 4.33 respectively. It can be observed that the soils from different sampling points gave relatively low and variable extractable nitrogen. Soils 1 and 2 had higher values compared with the other 4 soils.

Figures 4.34, 4.35 and 4.36 show the extractable-P, extractable-K and extractable-Mg respectively. The extractable-P shows some variability values within the times, while the extractable-K and extractable-Mg are much more uniform. Extractable-Mg is high across the site. The different points of the site have high pH and calcium carbonate leading to high extractable-Mg due to probable impurity of carbonate.



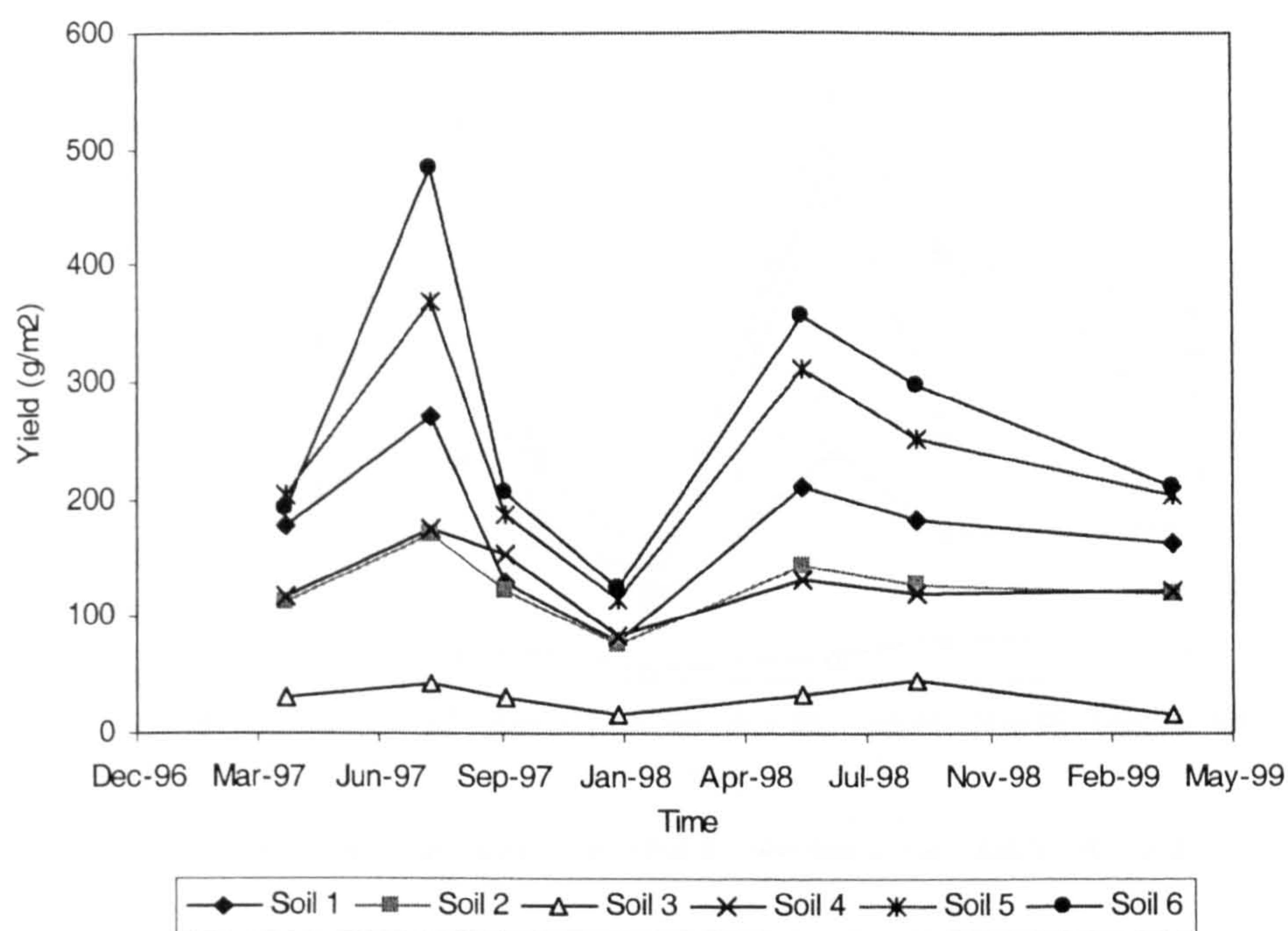


Figure 4.27: Yield of grass from 6 sampling points at different time.

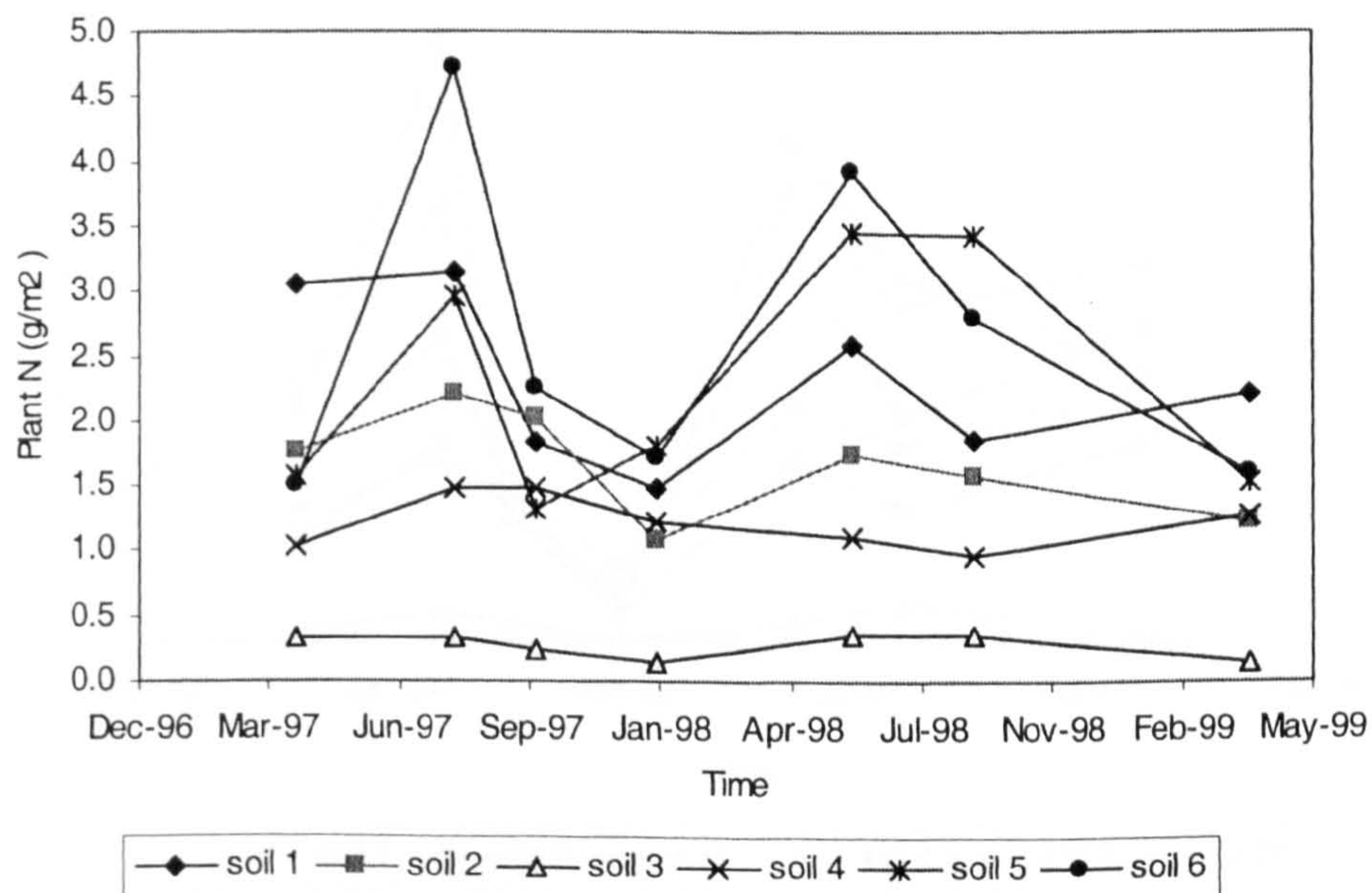


Figure 4.28: Amount of N in plant from 6 sampling points at different time.



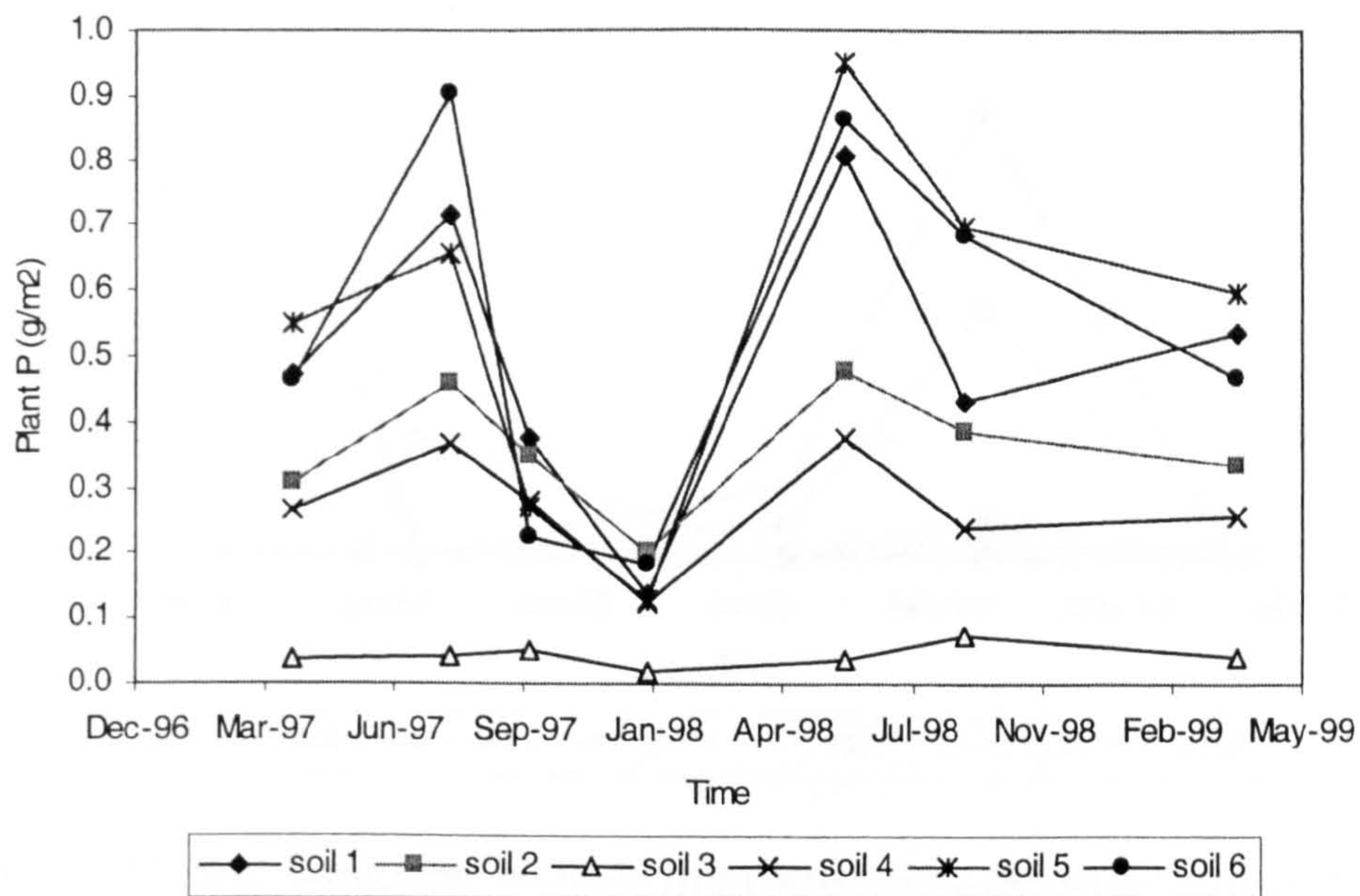


Figure 4.29: Amount of P in plant from 6 sampling points at different time.

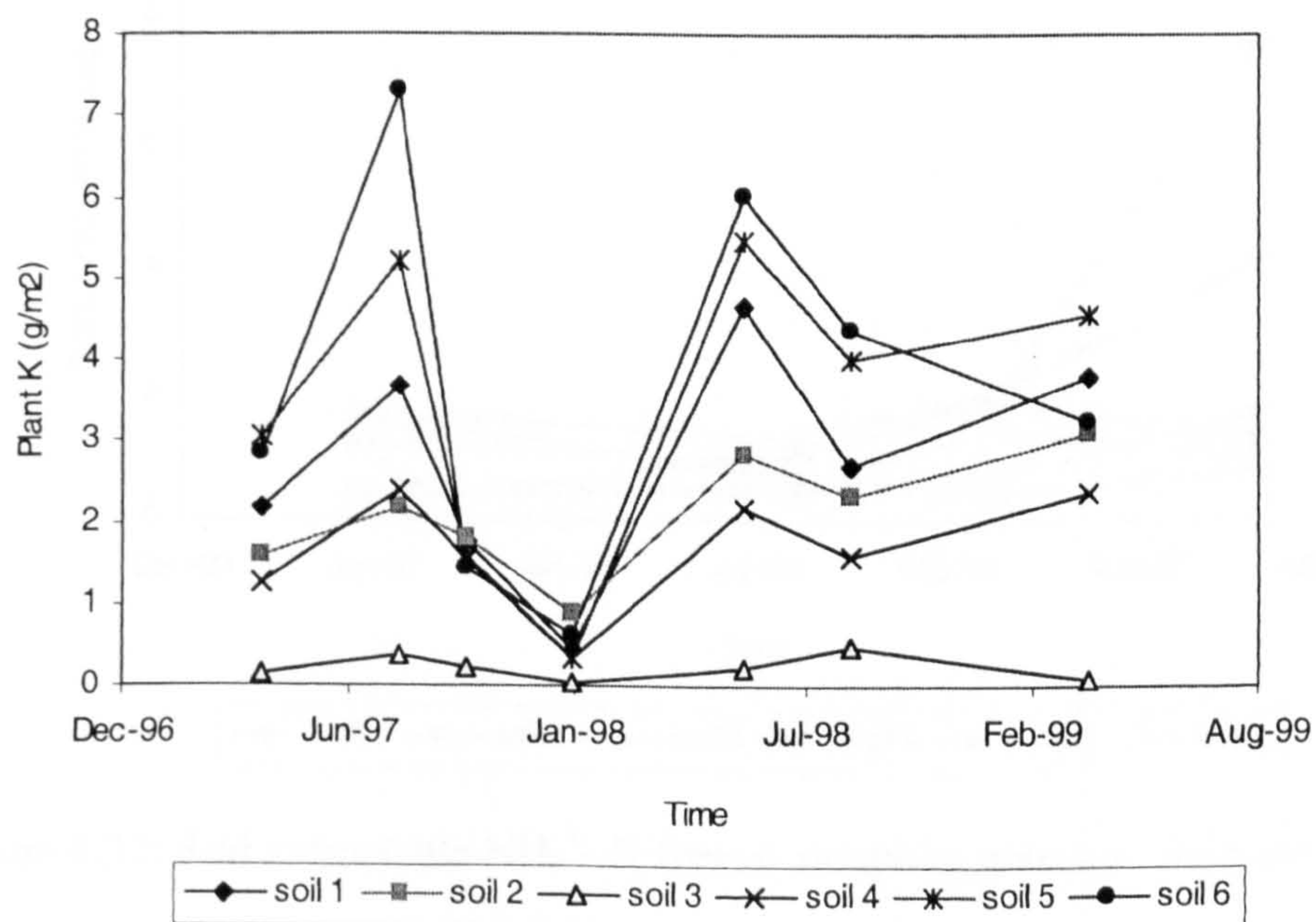


Figure 4.30: Amount of K in plant from 6 sampling points at different time.

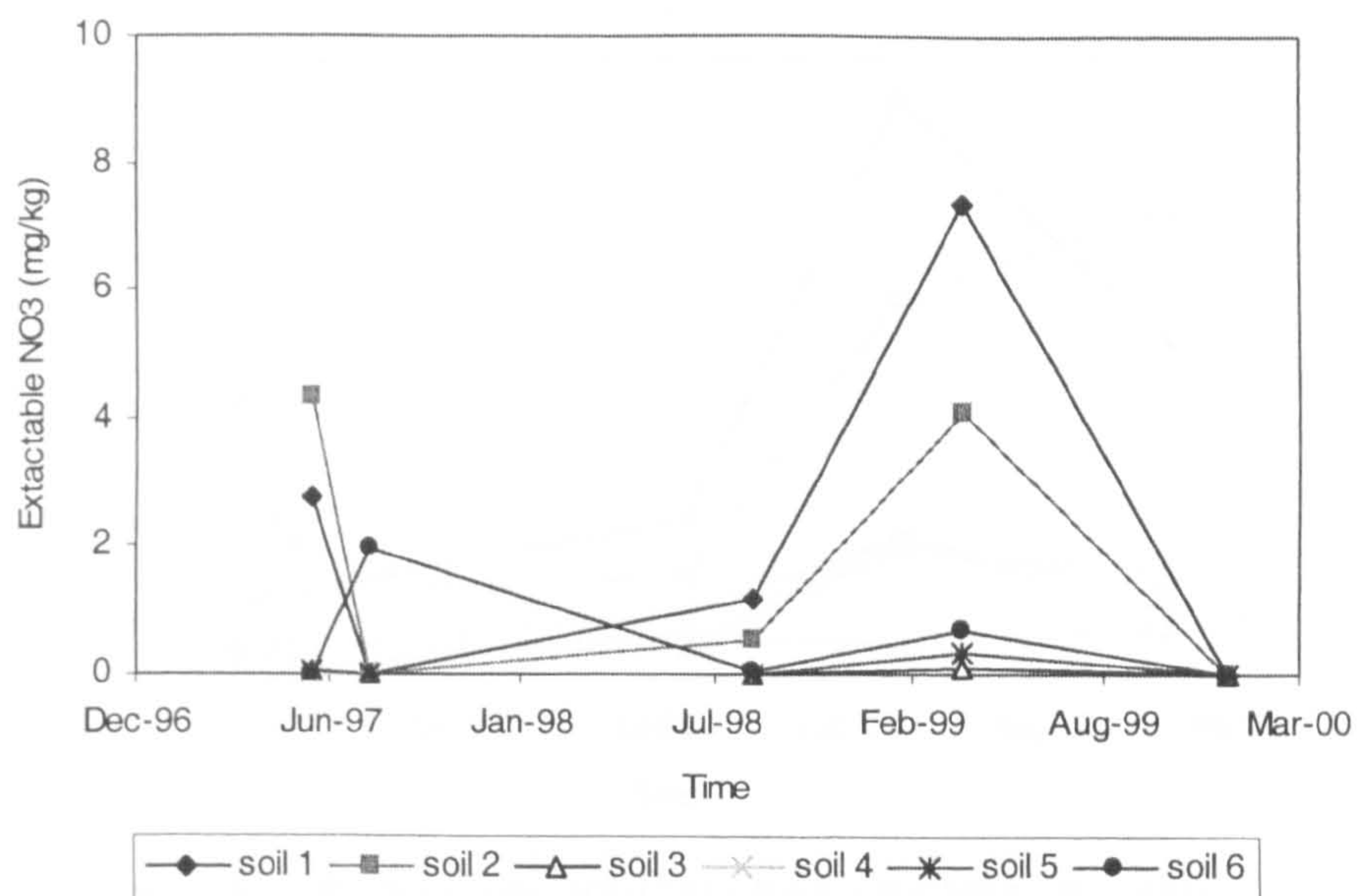


Figure 4.31: Soil extractable NO<sub>3</sub><sup>-</sup>-N from 6 sampling points at different time.

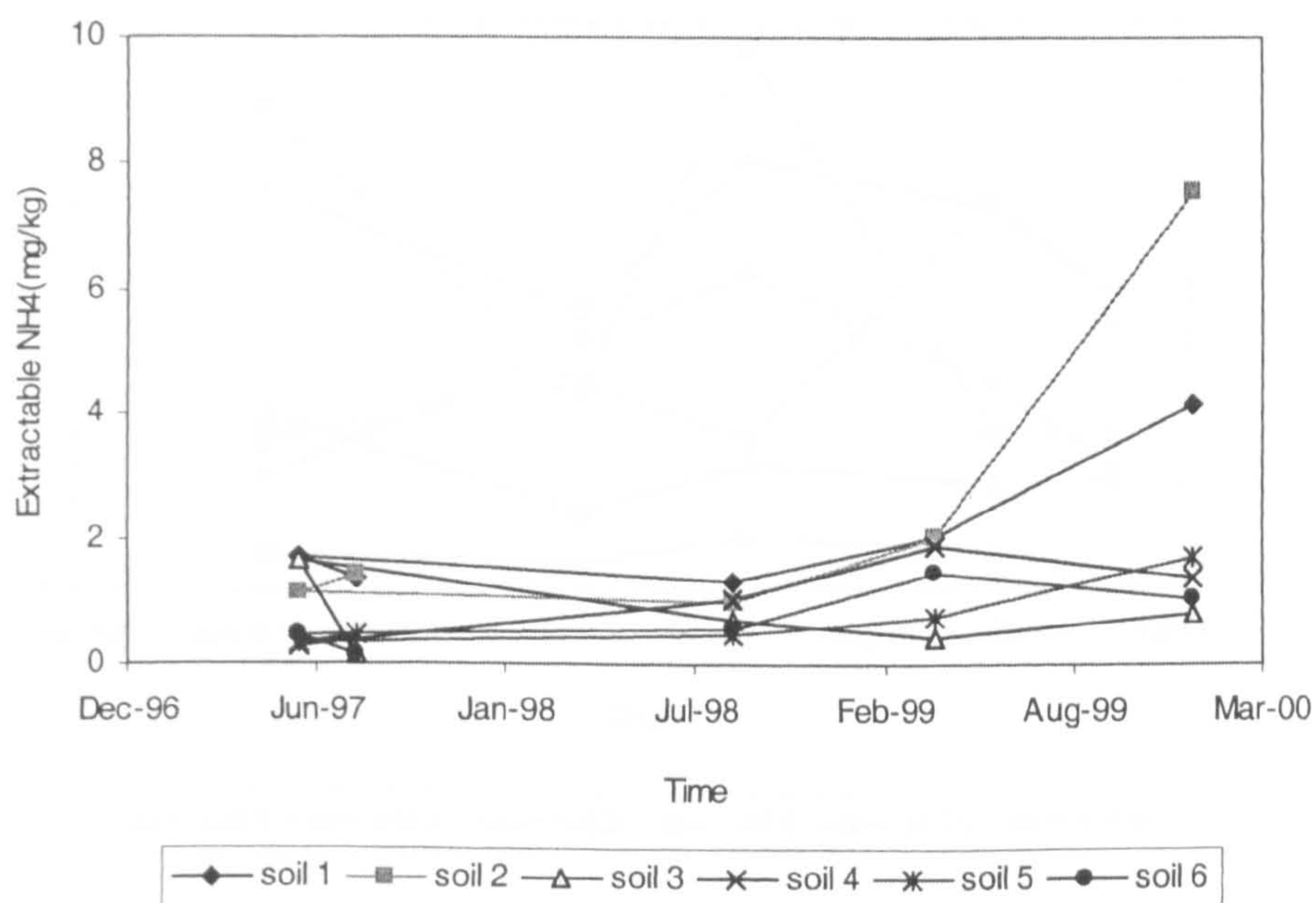


Figure 4.32: Soil extractable NH<sub>4</sub><sup>+</sup>-N from 6 sampling points at different time.



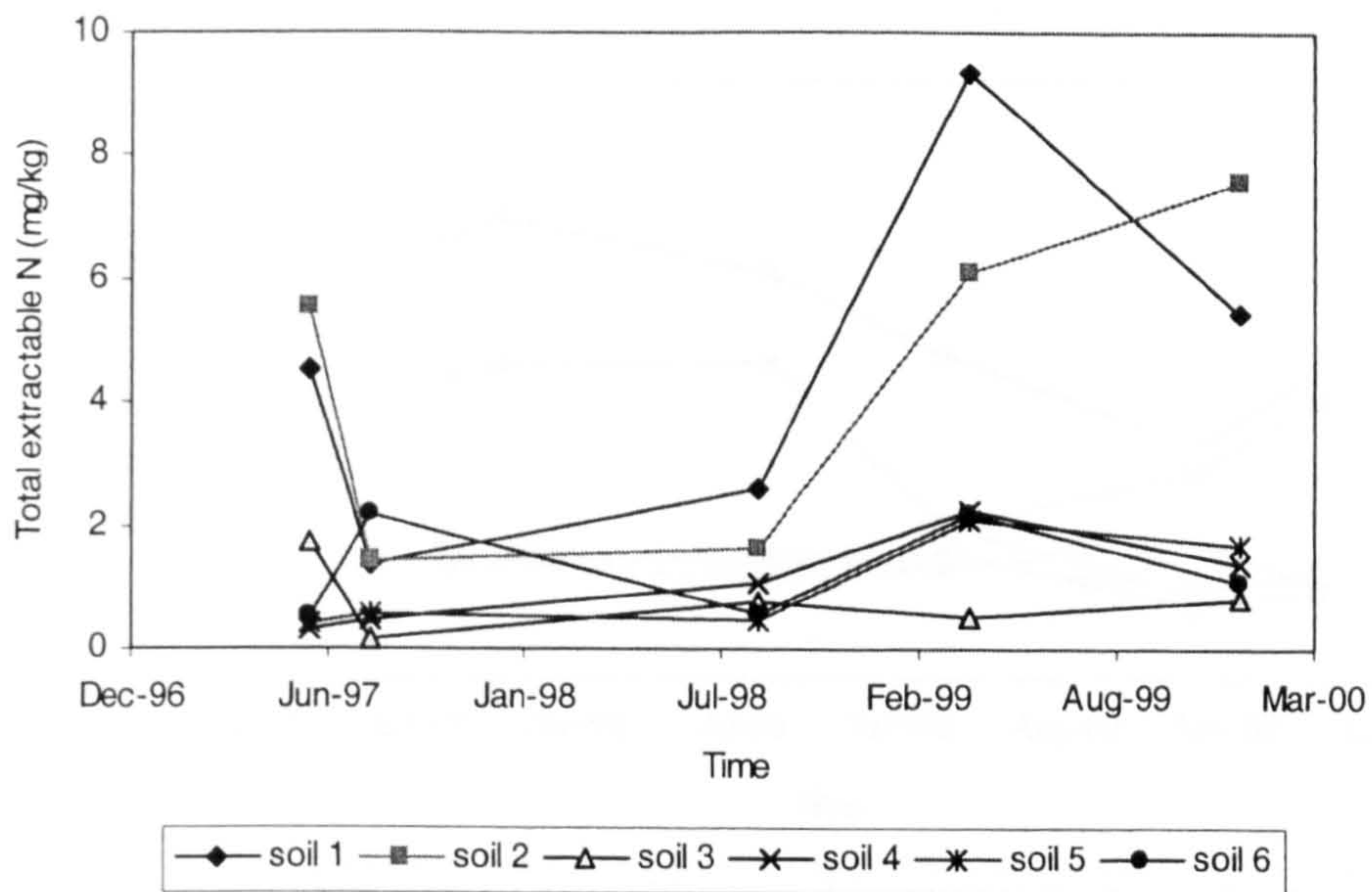


Figure 4.33: Soil total extractable-N from 6 sampling points at different time.

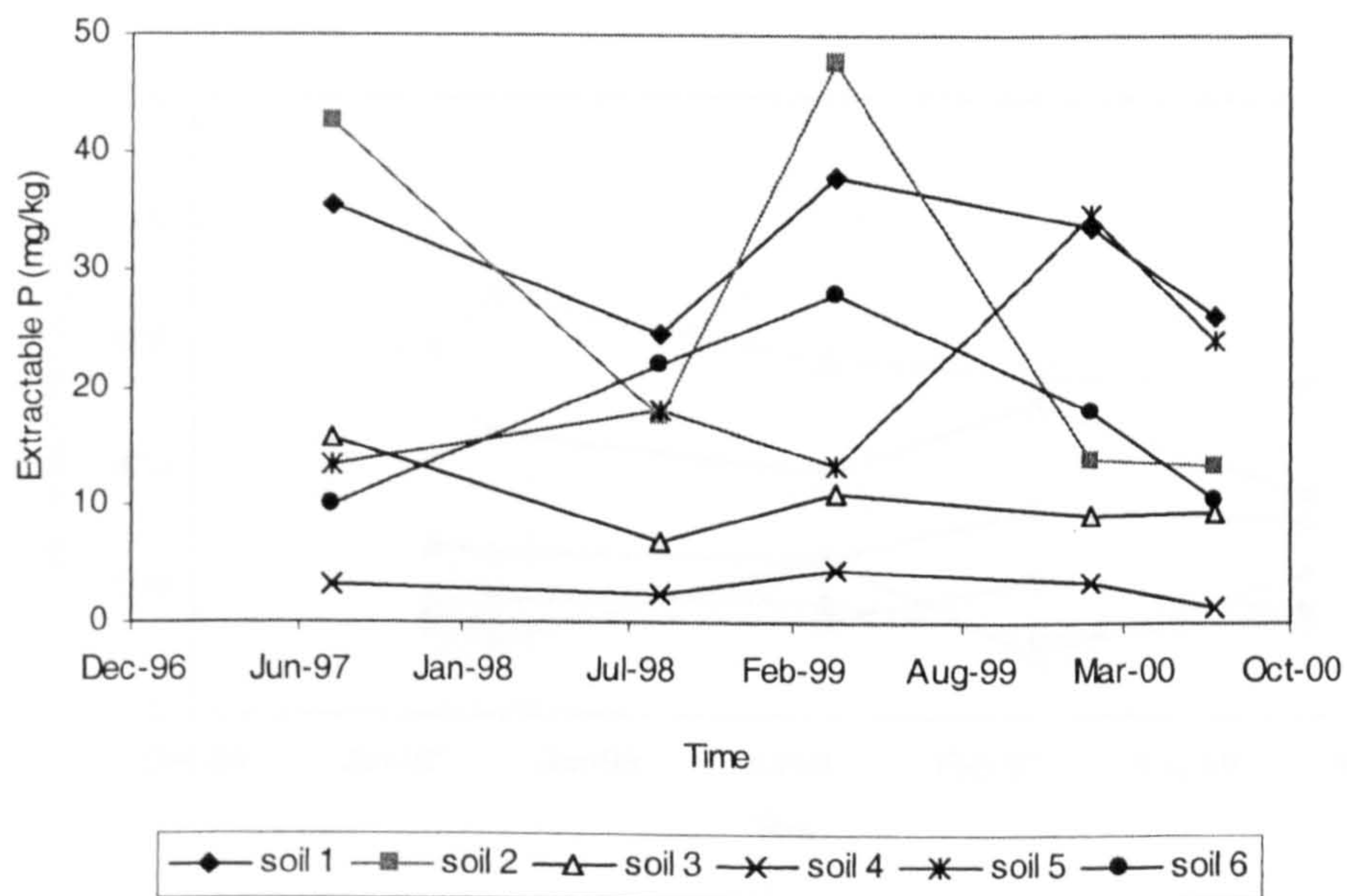


Figure 4.34: Soil l extractable-P from 6 sampling points at different time.

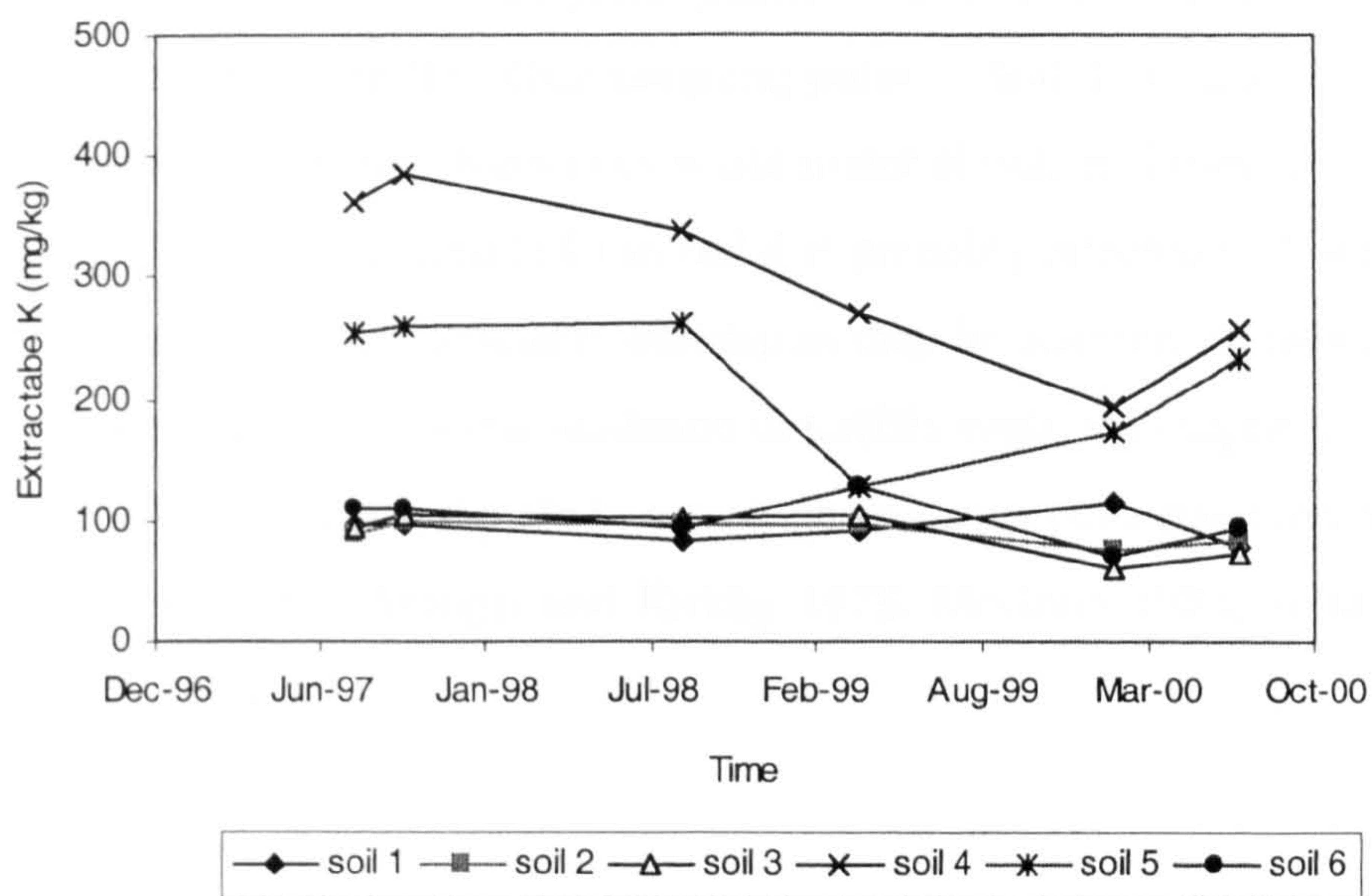


Figure 4.35: Soil extractable-K from 6 sampling points at different time.

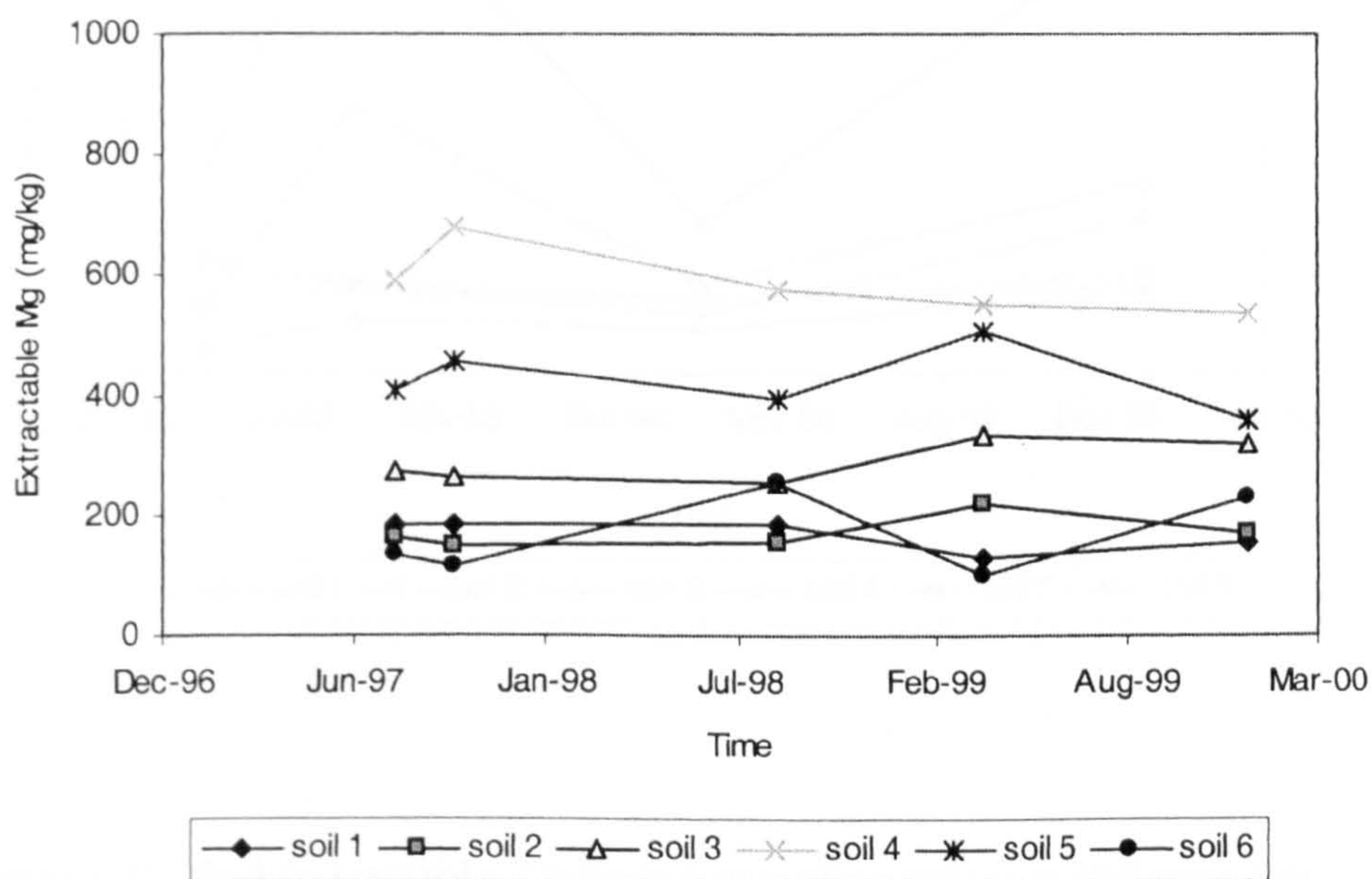


Figure 4.36: Soil extractable-Mg from 6 sampling points at different time.



Extractable Cr for four different times is shown in Figure 4.37. It can be observed that the surface soils from sampling points 3 and 4 have higher extractable chromium compared with the other sampling points. Soil 4 is located right at the bottom of the east slope which contains waste material rich in chromium at less than 30cm depth. The high extractable Cr in soil 4 is probably affected by leaching of Cr from the slope area. The extractable chromium may be affected by rainfall, as it is likely to be leached easily. Some oxidation of Cr(III) might also happened. Reducing of plant growth and yield in soils high in Cr has been observed by number of authors (Anon 1974, Mengel and Kirkby 1978, McGrath 1982, Salunkhe 1998 and Gimmler 1998).

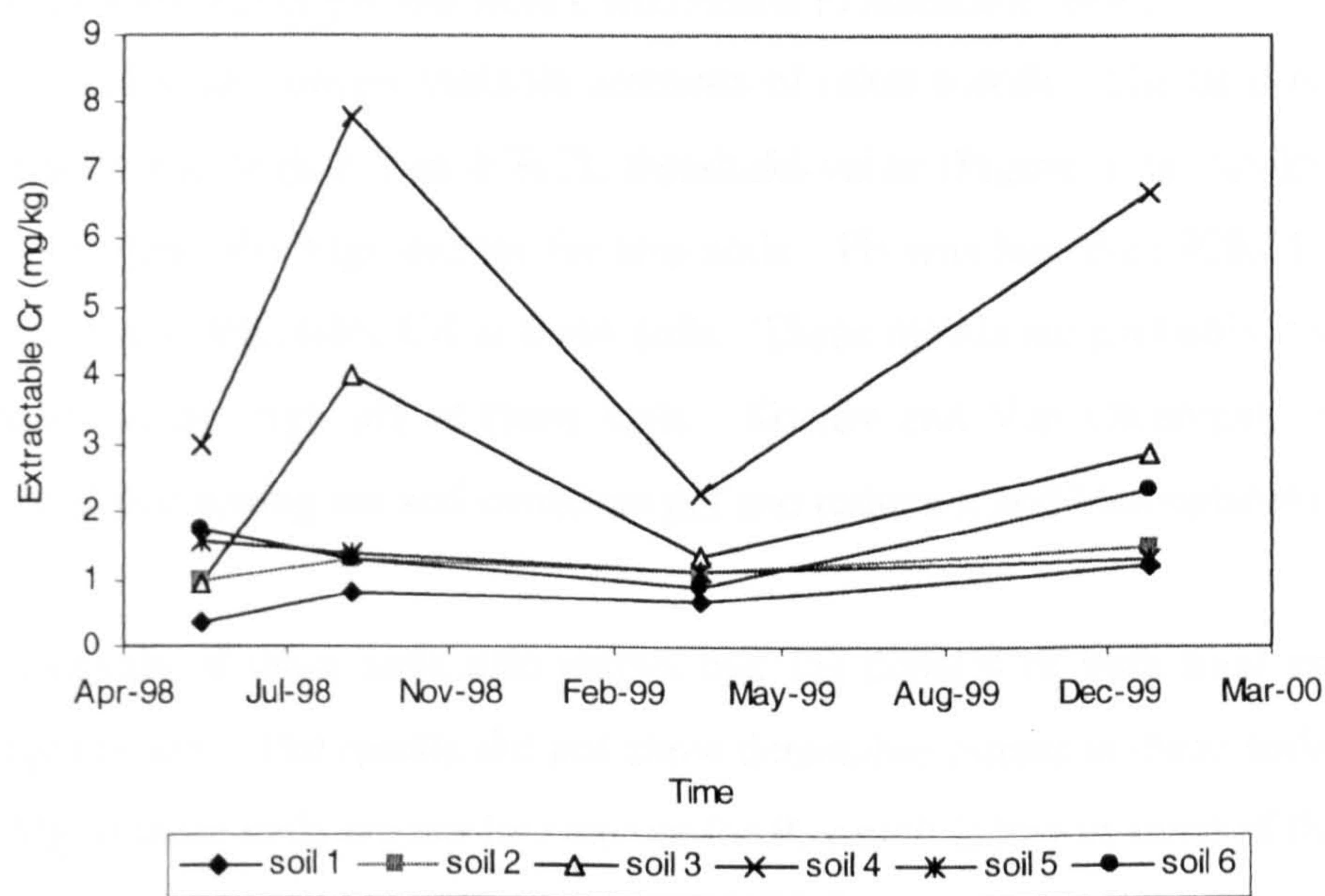


Figure 4.37: Soil Extractable–Cr from 6 sampling points at different time.



## 4.4 Conclusion

The soil samples from this site showed contamination with chromium. Soluble chromium is high in some soils especially on the slope part and is lower in some of the other soils. Water with a high level of chromium and a yellow colour can also be leached from the soils, especially from sub surfaces layers on the slope, and can increase the risk of the exposure of the chromium to the children and adults. Since the water soluble chromium, which is the Cr(VI) form, is low in some of these soils, especially the surface soils, it is assumed that the majority of this chromium is in the form of Cr(III). The pHs of these soils are high due to the large amount of carbonate. The solubility of the Cr(III) depends on the pH of the soil and is low in the higher pH. Therefore it can be assumed that the soluble Cr(III) in some of these soils is low. This is in agreement with finding of Bartlett and James (1988) that in the soils with higher pH and lime Cr(III) exist in insoluble form.

The soil samples contain variable amounts of other metals. The total-Ni content in these soils was higher than ICRCL threshold value (Figure 4.24) while Zn and Cu were not generally high except for two soils. Pb was less than ICRCL values and there was not detectable Cd in these soils. These metals are probably not accessible to plants at the high pH of these soils. Kostov and Van Cleemput. (2001) also observed that liming the soil increases pH and reduce metal bioavailability to plants.

The analysis of these soils also shows that the content of both total and available nitrogen is low. The results did not show detectable nitrate in these soils. The P, K and Mg in these soils are not low except for P, which is low in some of the samples.

Low growth of the vegetation on the soils can be because of lack of nutrients or toxicity of metals. There is low nitrogen and high chromium content of soils along with low vegetation growth, especially the sampling points on the slope area. Bradshaw (1983) expressed his view about plant growth for reconstruction of industrial contaminated land. He pointed out that in many cases these waste materials are very poor in nutrients, especially nitrogen. The content of other nutrients in this study, such as phosphorus, potassium and magnesium were not

generally low enough that they can probably be as limiting as nitrogen for growth of vegetation.

In the study to look at the seasonal changes in nutrients in the plants and exchangeable nutrients in the soils, it was observed that the nutrients and the yield in the plants change in different seasons in relation to growth of the plants. There was less change in some soil extractable nutrients with time at different sampling points. While some of the nutrients remained almost uniform.

This leads to a number of questions. Is poor vegetation caused by chromium toxicity or by lack of nutrients (nitrogen)? Is metal toxicity affecting the plant growth directly or indirectly by causing a problem in nutrient (nitrogen) turnover in the soils? Soils 3 and 4 had high extractable chromium, while the biomass yield of soil 4 is higher than soil 3. A number of experiments are necessary to look at the growth of the grass and its response to the nutrient fertilizers. Some additional experiments are also necessary in order to look at the nitrogen mineralization and nitrification in these soils.

## CHAPTER FIVE

### POT EXPERIMENT

#### 5.1 Introduction

Various factors are required for plant growth such as light, temperature, CO<sub>2</sub>, water, O<sub>2</sub> and mineral nutrition (Mengel and Kirkby 1978, Karrou and Maranville 1994 and Marschner 1999). The establishment and maintenance of vegetation as a part of the reconstruction of a contaminated or industrial land involves generation of functioning soil ecosystem containing adequate amounts of major nutrients (Bradshaw, 1983).

When studies were carried out for the site survey and the investigation of the seasonal pattern in nutrient levels of the plant and soils of the contaminated area, it showed a low growth of the vegetation especially the sampling points on the slope part of the site. It also indicated that the content of both total and available nitrogen in the soils is low. This brings the question about the nutrient status of the site. The soil samples from all parts of this site showed very high total Cr content. Reducing of plant growth and yield in soils high in Cr has been observed by number of authors (Anon 1974, Mengel and Kirkby 1978, McGrath 1982, Salunkhe 1998 and Gimmler 1998). Are low growth of the vegetation and the infertility of the site because of lack of the nutrients, or the metal toxicity?

The low level of extractable mineral nitrogen, together with the low vegetation biomass of the some soils implies that there might be a nutrient deficiency. Such assumptions suggest the need to investigate the effects of different fertilizers on the growth of vegetation of the soils of this site. Therefore it was decided to carry out two pot experiments for this purpose.



The objective of the first pot experiment was to apply different sets of fertilizers and observe which nutrient fertilizer may limit the plant growth. The objective of the second pot experiment was to see at which application rate the limiting nutrient can provide a good vegetation cover on this contaminated site. An attempt is also made to see the probable toxicity effect on plant growth.

In order to look at the response of the soil samples to the fertilizers and see which fertilizer is limiting the plant growth, 6 soil samples were chosen from the 6 sampling points of the site to represent different conditions of the site. They then were treated with the different combinations of the fertilizers N, P and K. This was achieved by conducting a pot experiment, which was carried out in the greenhouse. Ryegrass was used as a test crop that was grown in the soil samples and response was measured by dry matter yield.

Ryegrass is an easily manageable crop and gives a several cuts and high dry matter production. Its advantages compared with the other grasses or crops are in its well developed roots system and providing a good soil cover. Therefore it can prevent losses of nutrients by leaching and runoff. Because of its efficiency in utilizing applied fertilizers and nutrients under the greenhouse and field condition and its tolerance of variation in the nutrient balance, it is usually used and selected as the test crop. Further to the above reasons, since the climate condition of the area is suitable for the growth of grass this seed was used in this study (Khan, 1994).

After the discovery of a certain nutrient, which limits plant growth, a suitable application rate of that nutrient is needed to establish and maintain a good vegetation in the soils of the site. Therefore a further experiment would be necessary to investigate the plants response to different level of the limiting fertilizer. This can be obtained by conducting another pot experiment using same plant as a test crop in the 6 soils.

**5.2 Materials and Methods**

**5.2.1 First Pot Experiment**

**5.2.1.1 Design of the First Pot Experiment**

A random block design was used in this experiment. Six soil samples from 6 sampling points were used and four treatments were applied to each soil with 4 replicates for each treatment. Therefore there were 16 pots for each soil, which came out to 96 pots altogether. They were numbered and labelled according to the treatments and the replicates.

**5.2.1.2 Fertilizer Treatments**

The fertilizer treatments of soils were as follow:

<u>Treatment</u>	<u>N</u>	<u>P</u>	<u>K (kg/ha)</u>
1(A)		0	0    0
2(C)		100	0    0
3(D)		0	50   50
4(B)		100	50   50

**5.2.1.3 Layout for Pot Experiment**

The replicates of each fertilizer treatment for each soil are given in Table 5.1. The pots were placed in the saucers laid out in 4 blocks in the random block design, as shown in Table 5.2.

Soil No	Treatment	Fertilizer N/P/K (kg/ha)	Replicates			
1	1	0/0/0	1	2	3	4
	2	100/0/0	9	10	11	12
	3	0/50/50	13	14	15	16
	4	100/50/50	5	6	7	8
2	1	0/0/0	17	18	19	20
	2	100/0/0	25	26	27	28
	3	0/50/50	29	30	31	32
	4	100/50/50	21	22	23	24
3	1	0/0/0	33	34	35	36
	2	100/0/0	41	42	43	44
	3	0/50/50	45	46	47	48
	4	100/50/50	37	38	39	40
4	1	0/0/0	49	50	51	52
	2	100/0/0	57	58	59	60
	3	0/50/50	61	62	63	64
	4	100/50/50	53	54	55	56
5	1	0/0/0	65	66	67	68
	2	100/0/0	73	74	75	76
	3	0/50/50	77	78	79	80
	4	100/50/50	69	70	71	72
6	1	0/0/0	81	82	83	84
	2	100/0/0	89	90	91	92
	3	0/50/50	93	94	95	96
	4	100/50/50	85	86	87	88

Table 5.1: Replicates of each fertilizer treatment for each soil sample.



Row1	45	41	57	77	13	85	49	73	25	65	53	61
Row2	58	30	78	42	82	18	2	50	62	74	66	10
Row3	27	87	7	31	3	47	67	19	63	79	75	39
Row4	16	72	4	52	48	76	80	88	44	36	92	84

Continued ....

Row1	29	69	1	5	81	93	89	33	21	37	9	17
Row2	70	90	14	26	46	6	34	38	54	22	86	94
Row3	55	11	59	23	35	71	95	51	43	91	15	83
Row4	64	32	20	12	96	68	24	28	40	56	60	8

Table 5.2: The layout of the first pot experiment.

#### **5.2.1.4 Pot**

PVC pots with 10cm internal top diameter and 10cm height were used in this experiment.

#### **5.2.1.5 Soil Samples**

Six sampling points were chosen to represent the main sampling parts of the site. The samples were from the whole site based on the three parts of the site i.e. flat part on the top of the slope, the slope and the flat part at the bottom of the slop (Figure 4.11). Sampling point 1 was selected from the flat part of the top of the site, which had a good grass cover and some small birch trees towards the back and close to the railway embankment. The profile had loose ash material. Two sampling points (2 and 3) were chosen on the slope site. Sampling point 2 was from the planted part west half of the slope. This part had a dense stand of small birch trees with some grass and a loose ash profile with many tree roots. Sampling points 3 was located on the unplanted east side of the pathway on the slope part. This was very poorly vegetated with sparse birch trees. The profile contained loose ash with the Cr contaminated calcium carbonate waste material at less than 30cm depth. Three soil samples 4 to 6 were chosen from the flat part of the site at the bottom of the spoil, which had patchy grass. The profile had uniform ash material with very compact material below 15cm depth. Sampling point 4 was right at the bottom of the east side of the slope part and quite often was very wet. Sampling point 5 was at the center of the east part of the flat area and sampling point 6 was at the center of the west part of the flat area.

Soil samples were taken from 0-15cm. The surface vegetation dead leaves and the branches were removed from the topsoil and all soil samples were put in plastic bags, labeled and carried to the laboratory. About 10kg of each soil were then spread on the clean plastic sheet, the large aggregates were partly broken down. Each soil sample was

mixed thoroughly and was allowed to partially dry at room temperature. Then the soils were passed through a 10mm plastic sieve.

#### **5.2.1.6 The Analysis of Samples**

Soil samples were broken up to pass through a 2mm sieve for soil analysis. The pH and the percentage of the organic matter of the soils were measured as described in the section 2.1.3 and 2.1.9. The soils were extracted for inorganic nitrogen (ammonium-N, nitrite-N and nitrate-N), extractable phosphorus, extractable potassium, extractable magnesium and soluble chromium as described in the sections 2.2.1, 2.2.3, 2.2.4, 2.2.5 and 2.2.2 respectively. The extractable ammonium-N, extractable nitrite-N and extractable nitrate-N, extractable P, extractable K and extractable Mg were measured as described in sections 2.4.3, 2.4.5, 2.4.6, 2.4.7, 2.4.9 and 2.4.2 respectively.

#### **5.2.1.7 Making the Fertilizer Solutions**

##### **Treatment 1 [0/0/0 (N/P/K)]**

Deionized water was used for this treatment.

##### **Treatment 2 [100/0/0 (N/P/K)]**

14.8174g of the ammonium sulphate with the molecular weight of 132.13 was weighed and dissolved in deionized water. The volume was then made to 1 litre with deionized water. 25ml of this solution per pot is equivalent to 100kg/ha-N.

##### **Treatment 3 [0/50/50 (N/P/K)]**

8.04g of the sodium dihydrogen phosphate with the molecular weight of 156.01 and 3.05g of the potassium chloride with the molecular weight of 74.55 were weighed and



dissolved in deionized water. The volume was then made to 1 litre with deionized water. 25ml of this solution per pot is equivalent to 50kg/hect-P and 50kg/ha-K.

#### **Treatment 4 [100/50/50 (N/P/K)]**

14.8174g of the ammonium sulphate, 8.04g of the sodium dihydrogen phosphate and 3.05g of the potassium chloride were weighed and dissolved in deionized water. The volume was then made to 1 litre with deionized water. 25ml of this solution per pot is equivalent to 100kg/ha-N, 50kg/ha-P and 50kg/ha-K.

#### **5.2.1.8 First Growth Period**

An amount of partially dried of each soil sample equivalent to about 350g oven dried basis was weighed into each pot. More soils were weighed from the samples with the higher density.

An amount of 0.5g of ryegrass seed (*Lolium perenne*), variety: Merbo, reference number: DK. 759129, was sown on the surface of the soil of the each pot. They were covered with the black plastic sheet to prevent surface drying and to enhance the germination. It took about 3 days for the completion of the germination. Care was taken to avoid any dryness of the surface soils by watering them during this time. A volume of 25ml of the each fertilizer treatment was applied to the each pot. They then were transferred to the greenhouse.

The experiment was carried out in a green house at a minimum of 5-9°C with a mean of 8°C and a maximum ranging 12-25°C with a mean of 18°C temperature. A 16 hours supplementary light was applied to provide natural day length in March.

In order to establish a good growth of grass the pots were watered 2-3 times a week. The irrigation increased according to requirement in the later stages of the plant growth. Enough attention was made to ensure that the saucers did not become dried and the soils

remained moist throughout the experiment time since the water stress decreases nutrient uptake and dry matter yield (Karrou and Maranville, 1994). After 8 weeks the grasses had almost stopped growing. The watering was stopped 2 days before cutting in order to dry the soil partially and ease cutting the grass. The grass was cut just above the soil surface by stainless steel scissors and the yields were measured.

#### **5.2.1.9 Second Growth Period**

The same amounts of the fertilizers were applied to the pots again and they were irrigated and allowed to grow for another period of 8 weeks. They were then cut again and the amount of yield was measured. Soils with no treatment were used as the control, the same as it was done for the first harvest.

#### **5.2.1.10 Measuring the Dry Yield**

The fresh grasses were put in pre-weighed paper envelopes and were weighed again. They then were put in the oven at 80°C for 48 hours to be dried, and the dry yield was calculated. The data of the yields from the two harvests were combined to obtain the total dry yield of this experiment.

### **5.2.2 Second Pot Experiment**

#### **5.2.2.1 Design of the Second Pot Experiment**

A random block design was used in this experiment. Six soil samples from 6 sampling points were used and seven levels of N had been applied to the each soil with 4 replicates for the each treatment. Therefore there were 28 pots for each soil, which came to 168 pots altogether. They were numbered and labeled according to the treatments and the replicates.

**5.2.2.2 Fertilizer Treatments**

The fertilizer treatments of soils were as follows:

	<u>N</u>	<u>P</u>	<u>K</u>
	<u>kg/ha</u>		
A	0	50	50
B	25	50	50
C	50	50	50
D	75	50	50
E	100	50	50
F	125	50	50
G	150	50	50

**5.2.2.3 Layout for the Second Pot Experiment**

The replicates of each level of nitrogen for each soil are given in Table 5.3. The pots were placed in the saucers laid out in 4 blocks in the random block design, as shown in Table 5.4.



Soil No	Treatment	Fertilizer N/P/K (kg/ha)	Replicates			
1	A	0/50/50	1	2	3	4
	B	25/50/50	5	6	7	8
	C	50/50/50	9	10	11	12
	D	75/50/50	13	14	15	16
	E	100/50/50	17	18	19	20
	F	125/50/50	21	22	23	24
	G	150/50/50	25	26	27	28
2	A	0/50/50	29	30	31	32
	B	25/50/50	33	34	35	36
	C	50/50/50	37	38	39	40
	D	75/50/50	41	42	43	44
	E	100/50/50	45	46	47	48
	F	125/50/50	49	50	51	52
	G	150/50/50	53	54	55	56
3	A	0/50/50	57	58	59	60
	B	25/50/50	61	62	63	64
	C	50/50/50	65	66	67	68
	D	75/50/50	69	70	71	72
	E	100/50/50	73	74	75	76
	F	125/50/50	77	78	79	80
	G	150/50/50	81	82	83	84
4	A	0/50/50	85	86	87	88
	B	25/50/50	89	90	91	92
	C	50/50/50	93	94	95	96
	D	75/50/50	97	98	99	100
	E	100/50/50	101	102	103	104
	F	125/50/50	105	106	107	108
	G	150/50/50	109	110	111	112

Table continued .....

Soil No	Treatment	Fertilizer N/P/K (kg/ha)	Replicates			
5	A	0/50/50	113	114	115	116
	B	25/50/50	117	118	119	120
	C	50/50/50	121	122	123	124
	D	75/50/50	125	126	127	128
	E	100/50/50	129	130	131	132
	F	125/50/50	133	134	135	136
	G	150/50/50	137	138	139	140
6	A	0/50/50	141	142	143	144
	B	25/50/50	145	146	147	148
	C	50/50/50	149	150	151	152
	D	75/50/50	153	154	155	156
	E	100/50/50	157	158	159	160
	F	125/50/50	161	162	163	164
	G	150/50/50	165	166	167	168

Table 5.3: Replicates of each nitrogen level fertilizer for each soil sample.

Row1	125	69	1	141	113	157	77	41	49	109	89	97	9	5
Row2	54	90	70	166	78	58	138	82	66	110	42	158	2	18
Row3	27	131	67	59	15	7	95	31	23	19	79	87	83	99
Row4	164	116	96	64	88	28	12	84	40	136	124	60	24	32

Continues....

Row1	29	85	137	149	33	105	161	101	121	81	93	61	73	57
Row2	106	30	146	46	26	118	22	62	134	154	130	34	6	38
Row3	71	167	163	11	119	115	155	151	91	123	139	147	107	143
Row4	152	112	76	120	144	80	20	168	44	160	132	72	48	92

Continues....

Row1	145	13	165	37	17	53	129	133	117	25	65	21	45	153
Row2	122	14	10	126	150	94	142	86	50	98	162	114	74	102
Row3	35	43	3	51	159	63	47	55	75	103	111	127	135	39
Row4	140	108	8	104	100	68	52	128	148	56	4	16	36	156

Table 5.4: The layout of pots in the second pot experiment



#### **5.2.2.4 Pot**

The pots, which were used in this experiment, were the same as those used in the first pot experiment (5.2.1.4).

#### **5.2.2.5 Soil Samples**

About 20kg of each soil sample was taken from the 0-15cm depth of each 6 sampling points for this experiment. The soil sample preparation was the same as the first pot experiment (5.2.1.5).

#### **5.2.2.6 The Analysis of Samples**

Soil samples were broken up to pass through a 2mm sieve for soil analysis and same analysis was done as the first pot experiment (5.2.1.6).

#### **5.2.2.7 Making the Fertilizer Solutions**

##### **-Treatment A [0/50/50 (N/P/K)]**

0g of the ammonium sulphate, 8.04g of the sodium dihydrogen phosphate and 3.05g of the potassium chloride were weighed and dissolved in deionized water. The volume was then made to 1 litre with deionized water. 25ml of this solution per pot is equivalent to 0kg/ha-N, 50kg/ha-P and 50kg/ha-K.

##### **-Treatment B [25/50/50 (N/P/K)]**

3.7043g of the ammonium sulphate, 8.04g of the sodium dihydrogen phosphate and 3.05g of the potassium chloride were weighed and dissolved in deionized water. The

volume was then made to 1 litre with deionized water. 25ml of this solution per pot is equivalent to 25kg/ha-N, 50kg/ha-P and 50kg/ha-K.

**-Treatment C [50/50/50 (N/P/K)]**

7.4087g of the ammonium sulphate, 8.04g of the sodium dihydrogen phosphate and 3.05g of the potassium chloride were weighed and dissolved in deionized water. The volume was then made to 1 litre with deionized water. 25ml of this solution per pot is equivalent to 50kg/ha-N, 50kg/ha-P and 50kg/ha-K.

**-Treatment D [75/50/50 (N/P/K)]**

11.1130g of the ammonium sulphate, 8.04g of the sodium dihydrogen phosphate and 3.05g of the potassium chloride were weighed and dissolved in deionized water. The volume was then made to 1 litre with deionized water. 25ml of this solution per pot is equivalent to 75kg/ha-N, 50kg/ha-P and 50kg/ha-K.

**-Treatment E [100/50/50 (N/P/K)]**

14.8174g of the ammonium sulphate, 8.04g of the sodium dihydrogen phosphate and 3.05g of the potassium chloride were weighed and dissolved in deionized water. The volume was then made to 1 litre with deionized water. 25ml of this solution per pot is equivalent to 100kg/ha-N, 50kg/ha-P and 50kg/ha-K.

**-Treatment F [125/50/50 (N/P/K)]**

18.5217g of the ammonium sulphate, 8.04g of the sodium dihydrogen phosphate and 3.05g of the potassium chloride were weighed and dissolved in deionized water. The volume was then made to 1 litre with deionized water. 25ml of this solution per pot is equivalent to 125kg/ha-N, 50kg/ha-P and 50kg/ha-K.

### **-Treatment G [150/50/50 (N/P/K)]**

22.2261g of the ammonium sulphate, 8.04g of the sodium dihydrogen phosphate and 3.05g of the potassium chloride were weighed and dissolved in deionized water. The volume was then made to 1 litre with deionized water. 25ml of this solution per pot is equivalent to 150kg/ha-N, 50kg/ha-P and 50kg/ha-K.

### **5.2.2.8 First Growth Period**

The amount of soil, which was used in each pot, was the same as the first pot experiment. The same amount of ryegrass seed and the same germination method which was used in the first pot experiment applied in the second experiment too (5.2.1.8).

They were covered with the black plastic sheet to prevent surface drying and to enhance the germination. It took about 3 days for the completion of the germination. Care was taken to avoid any dryness of the surface soils by watering them during this time. A volume of 25ml of the each fertilizer treatment was applied to the each pot. They then were transferred to the greenhouse.

The pots were irrigated 3-4 times a week. The irrigation increased according to requirement in the later stages of the plant growth. Enough attention was made to ensure that the saucers did not become dried and the soils remained moist throughout the experiment time. After 6 weeks the grasses had almost stopped growing. The watering was stopped 2 days before cutting in order to dry the soil partially and ease cutting the grass. The grass was cut just above the soil and the yields were measured.



5.2.2.9 Second Growth Period

Like the first pot experiment (5.2.1.9) the same amount of the fertilizers were applied to the pots again and they were irrigated and allowed to grow for another period of 6 weeks. They then were cut and the amount of yield was measured.

5.2.2.10 Measuring the Dry Yield

The dry matter of the yield was measured as described in section 5.2.1.10.

5.3 Results and Discussion

5.3.1 First Pot Experiment

The results of the chemical properties of the soil samples used in the first pot experiment are presented in Table 5.5. They are the mean of 3 replicates.

Soil No	pH	OM %	Extractable (mg/kg)							
			NH <sub>4</sub> <sup>+</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	P	K	Mg	Cr. K <sub>2</sub> SO <sub>4</sub>	Cr. Water
1	7.91	7.96	0.98	0.1	7.7	24.7	83	185	1.7	0.53
2	8.07	7.68	0.88	0.1	2.3	17.6	98	156	3.1	0.63
3	8.24	5.99	0.35	0.06	ND *	6.7	102	256	4.6	1.02
4	8.29	4.09	0.42	0.07	0.17	2.3	337	575	8.2	2.8
5	8.2	6.49	0.30	0.06	0.01	18	261	392	3.1	0.76
6	8.22	7.33	0.34	0.07	0.22	21.9	93	253	3.0	0.73

Table 5.5: Some chemical properties of the soil samples used for the first pot experiment.

\*ND: Non detectable < 0.005 mg/l extract solution or 0.1 mg/kg soil.

As indicated in the table the available nitrogen in the soils is low. The results of the soil available P, K and Mg nutrients were compared with the indexes suggested in the MAFF/ADAS Book (1988). The available phosphorus varied between the indexes 0-2 and the available potassium between the indexes 1-2. The available Mg in the soils is comparable with the index 3 in some soils and index 6 in other soils. Therefore it was decided that the magnesium need not be added to the soils as a fertilizer for the pot experiment. Less P and K fertilizers applied to the soils compared with the nitrogen.

The first pot experiment was carried out to look at the response of plants to different sets of N, P and K fertilizers to determine which fertilizer is limiting the plant growth. In addition attempts were also made to compare the effect of nitrogen in both the presence and absence of added P and K.

The response was measured by dry matter production. The results for the plant growth of the first harvest after 8 weeks from the six soils and 4 fertilizer treatments are given in Table 5.6. The yields of the second growth period, which was cut 8 weeks later, are shown in Table 5.7. The combined result data from two growth periods are shown in Table 5.9. The values in the tables are the average of the 4 replicates and are in g/m<sup>2</sup>. Figures 5.1 and 5.2 show a view of greenhouse experiment and individual different treatments for the first pot experiment.

An analysis of variance for a factorial model was applied to these data. The Tukey HSD was calculated to compare the fertilizer treatments for each data obtained for each growth period of each soil (Table 5.6 and 5.7). The treatments with the same letter in column are not significantly different from each other ( $p > 0.05$ ).



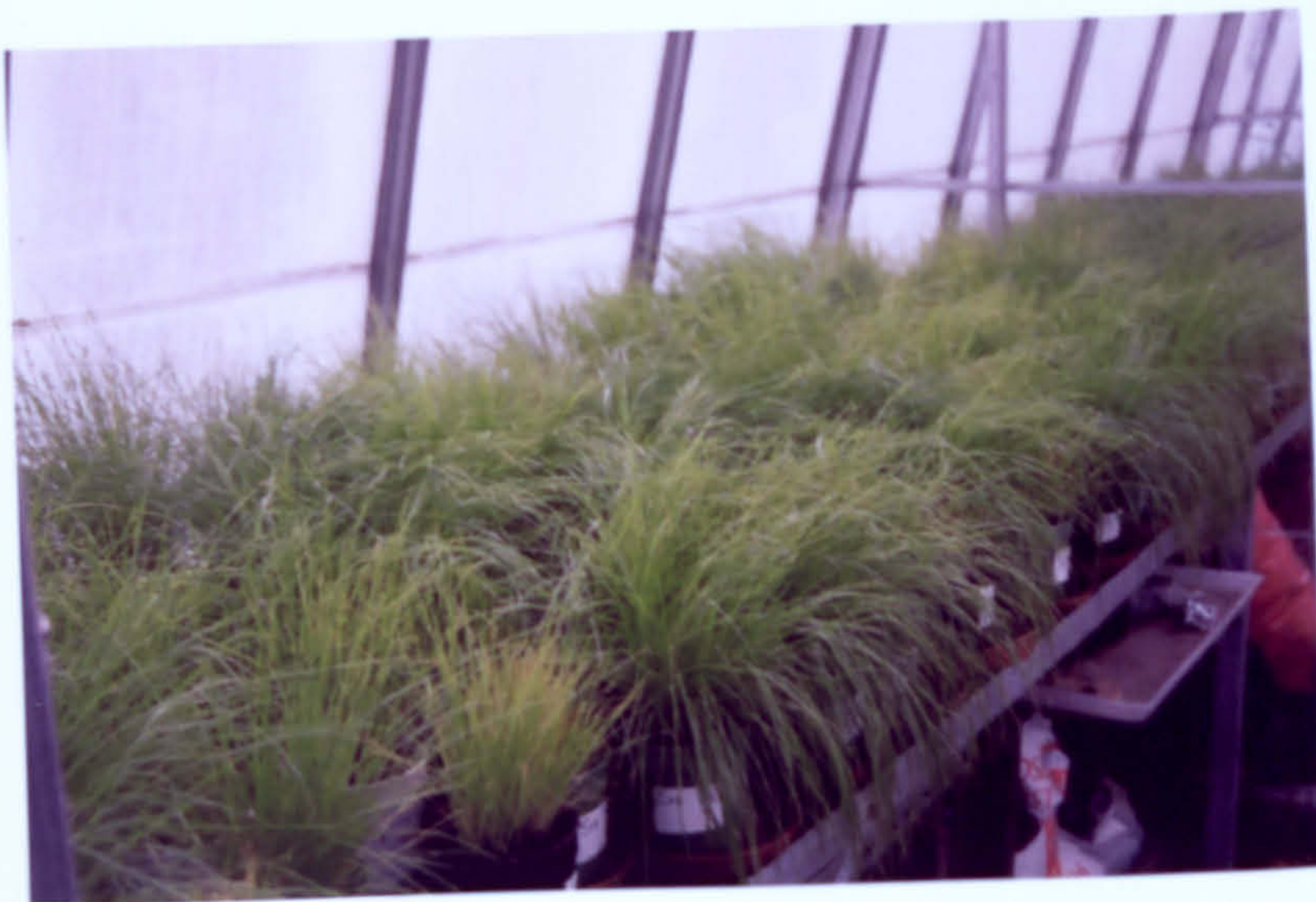


Figure 5.1 The overall view of the first pot experiment.



Figure 5.2 The comparison of the growth of grass with different combinations of N, P and K in the first pot experiment.



Treatment N P K (kg/ha)	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6	Overall Treatment
0 0 0	72 a	70 a	67 a	68 a	60 a	66 a	a
100 0 0	214 c	254 b	228 b	138 c	298 b	286 b	b
0 50 50	85 b	76 a	69 a	83 b	67 a	71 a	a
100 50 50	331 d	370 c	376 c	202 d	308 c	351 c	c

Table 5.6: The yield results of the first harvest (g/m<sup>2</sup>).

The values with the same letter in a column are not significantly different from each other (p>0.05).

Treatment N P K (kg/ha)	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6	Overall Treatment
0 0 0	34 a	33 a	23 a	22 a	10 a	22 a	a
100 0 0	250 b	274 b	256 c	116 c	283 c	331 b	b
0 50 50	33 a	37 a	20 a	19 a	18 b	23 a	a
100 50 50	481 c	475 c	496 d	287 d	437 d	461 c	c

Table 5.7: The yield results of the second harvest (g/m<sup>2</sup>).

The values with the same letter in a column are not significantly different from each other (p>0.05).

The yields for the control treatment show a generally poor growth for all soils Table 5.6 and 5.7. They had thin leaves during the two growth periods. The leaves were also yellow which is a symptom of nitrogen deficiency. Amongst the macronutrients nitrogen deficiency is a major factor limiting the growth of plants on the soils (Mengel and Kirkby (1978). The analysis of variance for a factorial model was applied to these data. The Tukey HSD was calculated to compare the control treatment for each growth period of each soil. The different letters in each column shows they are significantly different from each other and in the second period the values dropped dramatically (Table 5.8). This shows the nutrient reservoirs of all these soils are low. The different letter in each column shows values are significantly different from each other (p< 0.05).

Control Treatment N P K (0 0 0)	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6
First growth	72 a	70 a	67 a	68 a	60 a	66 a
Second growth	34 b	33 b	23 b	22 b	10 b	22 b

Table 5.8: The comparison of yield from the first and the second growth period for control treatment.

The values with the same letter in a column are not significantly different from each other ( $p>0.05$ ).

Table 5.9 shows the sum of two harvest yield. The analysis of variance for a factorial model was also applied to these data. The Tukey HSD was calculated to compare the fertilizer treatments. The treatments with the same letter in column are not significantly different from each other.

The values obtained with the P and K addition treatment, is almost the same as those obtained with the control. Their yield also reduced in the second growth period, which can be noticed from the Tables 5.6 and 5.7.

Treatment N P K (kg/ha)	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6	Overall Treatment
0 0 0	106 a	103 a	90 a	90 a	70 a	88 a	a
100 0 0	466 c	529 b	484 b	254 b	582 c	617 b	b
0 50 50	118 b	113 a	87 a	98 a	85 b	94 a	a
100 50 50	813 d	845 c	873 c	488 c	745 d	811 c	c

Table 5.9: The yield results of the (sum of two harvests) ( $\text{g/m}^2$ ).

The values with the same letter in a column are not significantly different from each other ( $p>0.05$ ).

The obtained values for the nitrogen only treatment and treatment with N, P and K fertilizer application in soil 4 is the lowest compared with the other soils (Tables 5.6,



5.7 and 5.9). This sampling point contains more available Cr than others. Moreover the soil was more compact and hard compared with the other soils. The soil was wet at the time of sampling. The question is whether there is a possibility of Cr toxicity affecting the yield in this sampling point.

The results of the sum of two growth period (Table 5.9) show that all these soils had a good response to nitrogen fertilizer. Treatment with nitrogen only, had a significantly higher yield compared with the control or P and K added treatment. The results of N, P and K treatment had significantly the highest yield in this experiment. These results confirm nitrogen is the limiting factor for growth of the grass in these soils and when nitrogen is applied at high rate without P and K, then P or K becomes the next limiting factor (*law of diminishing yield increment*, Mitscherlich, 1954 and Marschner, 1999). Therefore when N, P and K fertilizers are applied the yield is the highest.

### **5.3.2 Second Pot Experiment**

From the first pot experiment it was observed that nitrogen limits the plant growth in these soils. Nitrogen is one of the major mineral nutrients required for growth and development of plants (Mengel and Kirkby 1978, Karrou and Maranville 1994). Attention should be paid to the shortage of nitrogen in soil material in industrial land and build up adequate available nitrogen in order to handle the problem in these areas for reconstruction (Bradshaw, 1983). Alleviating the limiting factor results in a yield increase. The trend of this increase cannot be fully applied to all type of soils and spoils due to differences in nature of the material. Therefore a suitable application rate of this nutrient was necessary to ensure an adequate supply of nitrogen to establish and maintain a good vegetation in the soils of this site. Consequently the second pot experiment was carried out to look at the response of the vegetation to addition of the different rates of nitrogen fertilizer.

Since the soils were sampled at a different time for the second pot experiment they were analyzed again for pH, organic matter content and extractable nutrients and Cr in order



to look at the possible change or sampling variability in different time. The results of chemical properties of soil samples used in the second pot experiment are presented in Table 5.10. The results are the mean of 3 replicates.

Soil No	pH	OM %	mg/kg							
			Ext. NH <sub>4</sub> <sup>+</sup> -N	Ext. NO <sub>2</sub> <sup>-</sup> -N	Ext. NO <sub>3</sub> <sup>-</sup> -N	Ext. P	Ext. K	Ext. Mg	K <sub>2</sub> SO <sub>4</sub> Ext. Cr.	Water Ext. Cr.
1	7.5	7.96	4.3	ND *	1.1	24.3	78	152	3.7	1.20
2	7.8	7.72	7.5	ND	ND	13.3	85	170	4.1	1.47
3	8.1	5.68	0.8	ND	ND	9.4	74	317	4.9	1.87
4	8.3	3.74	1.4	ND	ND	1.2	256	537	20.3	6.67
5	8.2	6.49	1.7	ND	ND	24.1	231	356	3.9	1.27
6	8.1	7.31	1.1	ND	ND	10.4	94	230	7.4	2.33

Table 5.10: Some chemical properties of the soil samples used for the second pot experiment.

\*ND: Non detectable < 0.005 mg/l extract solution or 0.1 mg/kg soil.

As indicated in the table the level of available nitrogen, especially NO<sub>3</sub><sup>-</sup>-N and NO<sub>2</sub><sup>-</sup>-N in the soils were non detectable. There was some higher available NH<sub>4</sub><sup>+</sup>-N in the soil compared with the first pot experiment, which had not converted to nitrate. Like the first pot experiment, the results of soil available nutrients in soils from this sampling were compared with indexes suggested in MAFF/ADAS Book (1988). The available phosphorus varied between the indexes 0 and 2 and the available potassium between indexes 1 and 2. The available Mg in the soils was comparable with the indexes 3 in some soils and the index 6 in other soils. Therefore it was decided that magnesium was not added to the soils as a fertilizer for this pot experiment either. In order to see the real effect of different levels of the nitrogen fertilizer application it was decided to apply the same rate of the P and K fertilizer as they were applied in the first pot experiment as a background application. The soils had higher available Cr. In spite of the fact that care was taken in the selection of the sites the presence of the sampling variability indicates the heterogeneous nature of the waste material. The higher available Cr may

also be due to the timing of the sampling with respect to lower rainfall or partly oxidation of chromium(III). Overall the differences in the results are mostly because of sampling variability.

The results of the yield from the seven treatments of different levels of the nitrogen fertilizer are given in Figures 5.3, 5.4 and 5.5. Figure 5.3 represents the yield of the first growth period and Figure 5.4 shows the yield of the second growth period. The sum of the two yields is also demonstrated in Figure 5.5. Figures 5.6 and 5.7 show a view of greenhouse experiment and individual different treatments for the second pot experiment.

As shown in Figures 5.3, 5.4 and 5.5, the yield increases with the increase of the rate of nitrogen fertilizer. This increase is rapid with nitrogen application from 0 to 100kg/ha in all soils except for soil 4. There is very little change in yield by increasing the rate of nitrogen application over 100kg/ha. Since the K in these soils are not low, P may be the next limiting factor for growth. The nitrogen response curve for soil 4 shows increase in dry matter production with nitrogen rate application up to 50kg/ha. There is also a very little change in yield by increasing the rate of nitrogen application over 50kg/ha. The different response for soil 4 with the other soils to the nitrogen application can be seen in the same pattern in both harvests.

In a growth study which was carried out by Douglas and Crawford (1993) they applied different rates of nitrogen fertilizer (0, 50, 100 and 150kg/ha) for ryegrass growth. They observed that the obtained yield is higher with more nitrogen application. Their results showed a lower rate of yield increase at more than 100kg/ha nitrogen application. These researchers studied the effect of different level of compaction on recovery of yield in different rate of nitrogen application. They observed that the dry matter yield is lower in more compact soil for each nitrogen rate and contributed the lower yield to poor soil physical condition. They concluded that the soil condition resulting from compaction were alone sufficient to limit productivity without implicating possible contribution from plant damage.

The control for this experiment, which was the P and K, added treatment without nitrogen showed a higher result in the first growth period compare with same treatment of the second period for all soils. This treatment had actually the same fertilizer treatment as the treatment 3 in the first pot experiment.

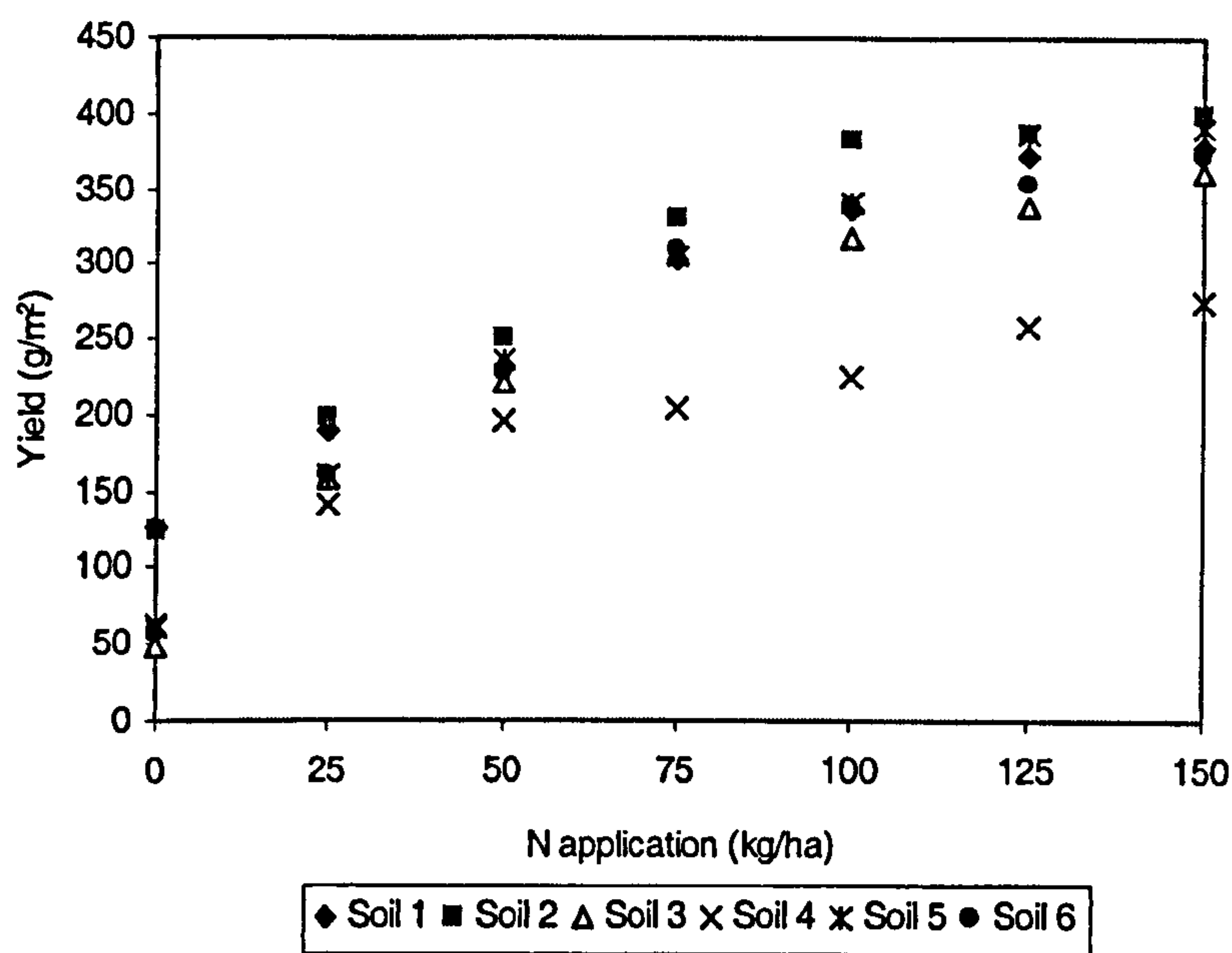


Figure 5.3: Response curve for the first harvest of the second pot experiment (g/m<sup>2</sup>).



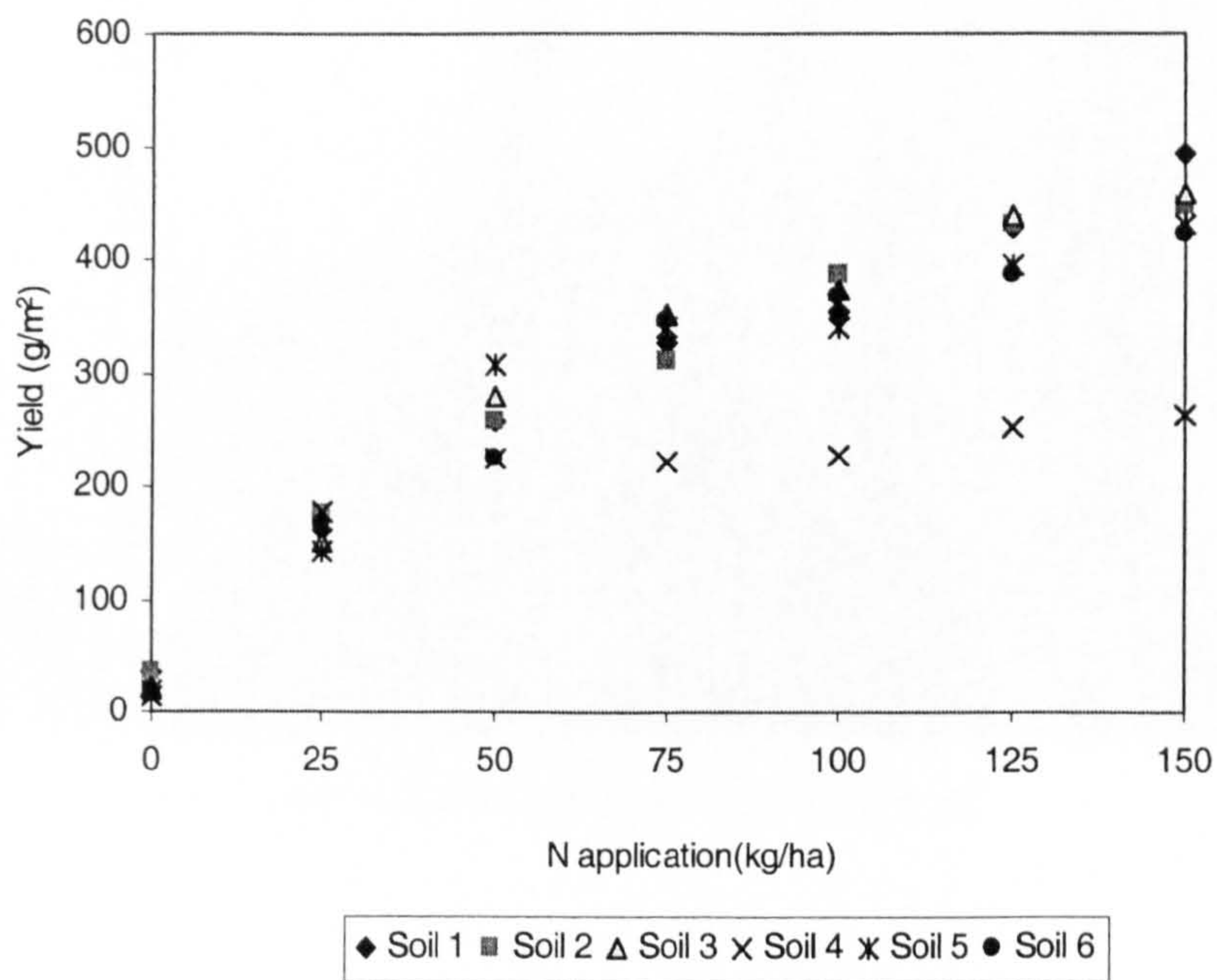


Table 5.4: Response curve for the second harvest of the second pot experiment (g/m<sup>2</sup>).

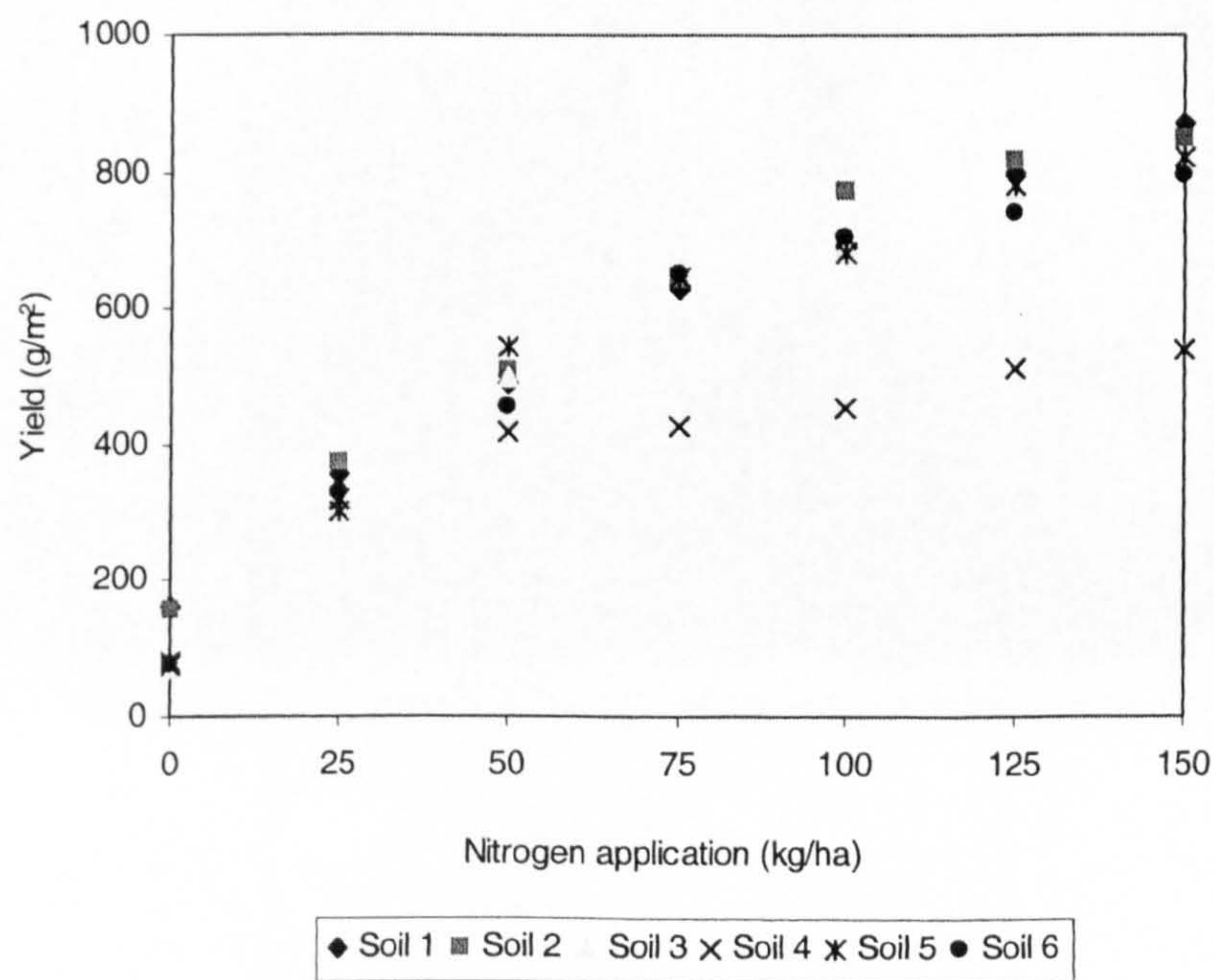


Figure 5.5: Response curve of sum of two harvest of the second pot experiment (g/m<sup>2</sup>).



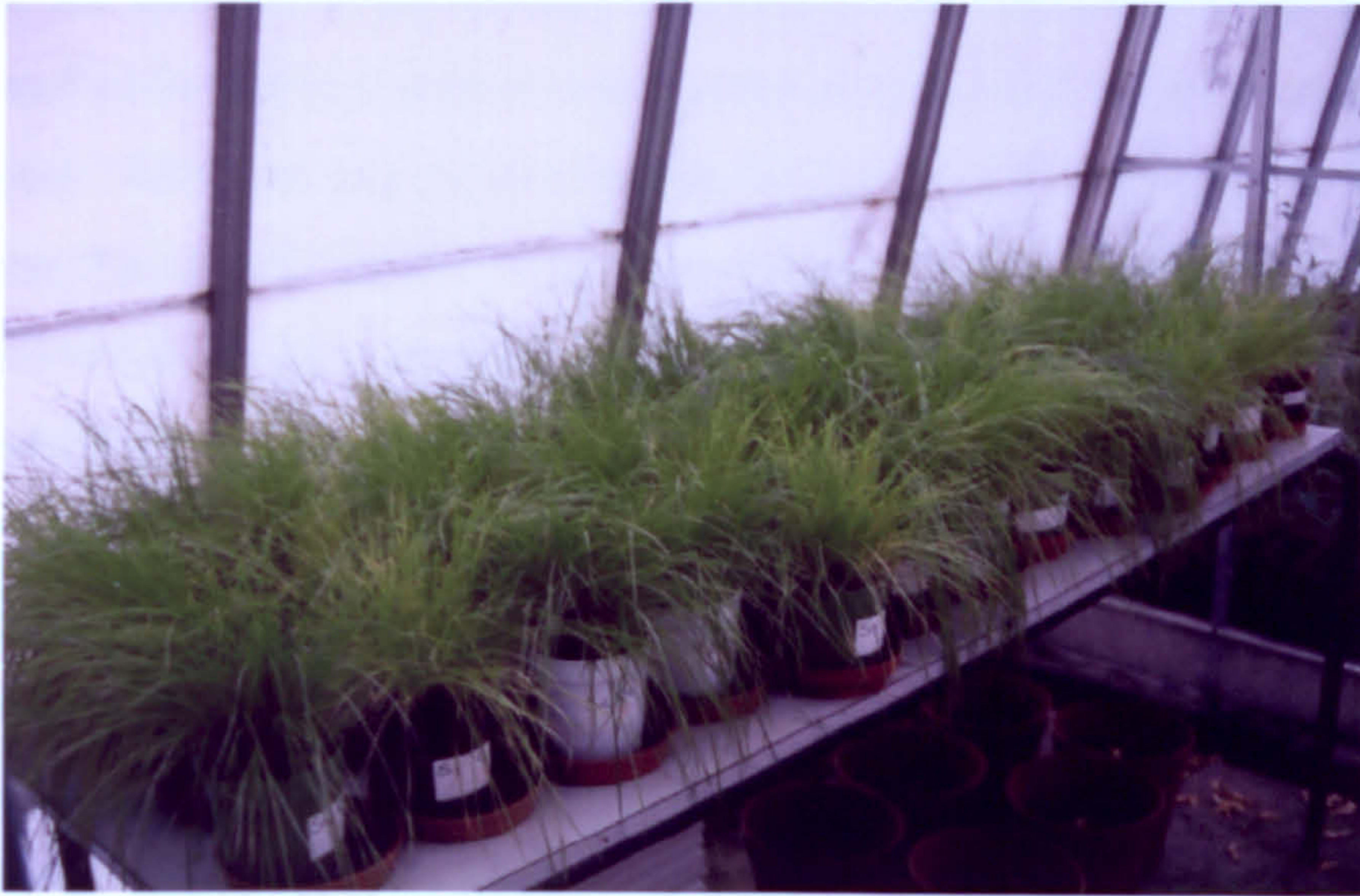


Figure 5.6: The overall view of the second pot experiment.

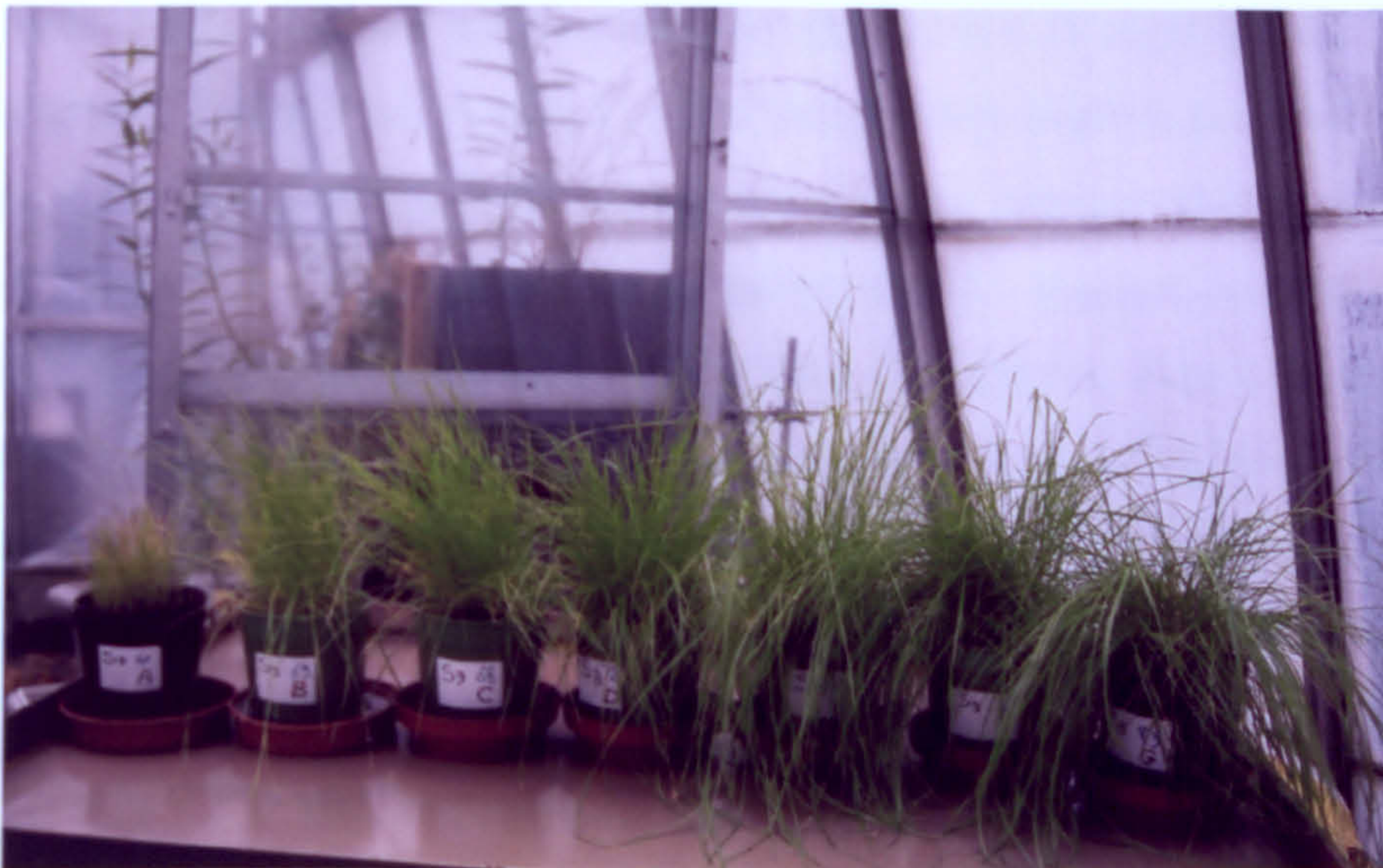


Figure 5.7: The comparison of the growth of grass with the different rates of nitrogen fertilizer.



After the addition of the nitrogen fertilizer to the soils the amount of yield obtained at the end of the harvest session was higher in the second harvest compared with the first harvest. Since the experiment was carried out in summer time the higher temperature during the second period of growth may affect the obtained yield. Moreover the remaining of nitrogen from the first application may also affect the growth in the second growth period.

Greenhouse trials were carried out by Sykes et al., (1981) to grow crops in soils containing Cr(III). They observed from the result that the presence of Cr(III), at the highest value which was 1000mg/kg in their study did not of itself have a toxic or inhibitory effect on plant growth. They pointed out that in their experiments nitrogen content was found to be limiting the growth not heavy metals. In the present study soils contains more than 2000mg/kg total Cr while in some soils like soil 4 this value is more than 10000mg/kg.

In soils high in heavy metals, biomass production is significantly inhibited by the presence of some heavy metals. The factors that control the total and bioavailable concentrations of heavy metal in soils are of great concern with regard to both human toxicity and biomass production (Alloway, 1995). Bartlett and James (1979) found severe damage of mustard, barley and alfalfa with Cr(VI). Due to much lower available Cr(VI) in the soils in present study probably the majority of the total chromium in these soils is in the form of Cr(III). Since the solubility of the Cr(III) depends on the pH of the soil, it can be assumed that the soluble Cr(III) in these soils is also low.

Vago et al., (1996) conducted a pot experiment to study the effect of Cr, N and on ryegrass growth. He used 80 and 160mg/kg nitrogen fertilizer treatments with using 25 and 75mg/kg Cr(III) as heavy metal treatment. He also found that the plant yield increase with increasing the nitrogen rate application and the dry matter production has not been affected by Cr concentration.



Salunkhe (1998) referred to Anon (1975) that in soil contaminated with between 5-60mg/kg Cr(VI) plant growth was reduced due to root damage. Weber and Day (1996) investigated the effect of nitrogen fertilizer on grass roots. They observed that the root length density increases with nitrogen fertilizer and this increase is higher in 0-15cm depth than the other depth. Distribution of root system is an important factor for ability of plant to uptake nutrient and water especially in nutrient limited ecosystem. They pointed out that the altering the most limited nutrient resource i.e. nitrogen elicit the stronger response from the roots.

The extractable Cr of the soils which were used for the second pot experiment was higher than those of soils which were used in the first pot experiment (Tables 5.5 and 5.10). The yield results of 100/50/50kg/ha N, P and K added treatments of the two experiments are given in Table 5.11. Comparing the extractable Cr and obtained yield between soil 3 and 4 in the first pot experiment show that the value of the extractable Cr in soil 4 is twice as much of the soil 3 and the yield of the soil 4 is about half of the that for soil 3. On the other hand comparison the yield and extractable Cr for soil 4 for the two pot experiments show that although the extractable Cr in soil 4 for the second pot experiment is almost 3 times more than that of the first pot experiment, the reduction of obtained yield is less. The difference between the two experiments is too small to be obtained as the effects of Cr level only (Table 5.11).

Treatment N P K (100/50/50)kg/ha	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6
First Pot Experiment	813	845	873	488	745	811
Second Pot Experiment	691	769	689	454	682	705

Table 5.11: The yield obtained with 100/50/50 (kg/ha) fertilizer in two pot experiments. (From Table 5.9 and Figure 5.5)

There are also other heavy metals, which can have toxicity effect on plant production. Ye *et al.* (1997) studied the effects of Zn, Pb and Cd toxicity on growth of *Typha latifolia* and compared it with non contaminated solution culture. They found that the shoot and root dry matter significantly reduced at 5mg/l Zn in culture solution. They also noticed that the length of the longest leaf and longest root were reduced in the 1-5mg/l Zn. In their experiment they observed that the elongation of plant roots was affected in the concentration of 10-20mg/l Pb culture solution. In case of cadmium they found severe toxic effects on plant growth and reduction of dry matter in 0.25 and 0.50 mg/l Cd. Alloway (1995) reported toxicity effect of Cd on number of plants like spinach, soybean, lettuce. He also pointed out the stunted growth or death of plant in high concentration of Pb.

Lombi *et al.* (1998) conducted a pot experiment and studied the effect of combined different metals in different level of addition on sunflowers (*Helianthus annuus L.*). They used 300mg/kg Zn, 100mg/kg Cu, 50mg/kg Ni, 50mg/kg V and 3 mg/kgCd for level 1 and two another levels with twice and triple amount of above concentrations on a metal deficient soil for their experiment. They observed that with the first level of metal addition the yield increased while after the second and the third levels the yield of sunflowers reduced due to metal toxicity.

There is also report of Mn toxicity on soya bean, cotton, tobacco etc. in range of 80-5000 mg/kg and acidic and warm climate (Alloway, 1995). Se is also toxic to plants in high concentration especially in form of selenate.

Vago *et al.* (1996) studied the effect of Ni, Cr concentration and lime on plant growth. As it was mentioned earlier they did not find Cr toxicity on plant. While they found that Ni cause a depression on the dry matter yield. They also noticed that the addition of calcium carbonate reduced the effect of Ni on plant growth and that is because lime increases the soil pH.



Different metals have different distributions in plants. Heavy metals like Ni, Cd, Zn, B, Mo and Se are recognized as being elements which are readily translocated to plant top after absorption through the root while Cr, Cu and Pb absorbed by plant remain in the root tissue (Alloway, 1995).

## 5.4 Conclusion

From the preliminary pot experiment, which was done to look at the effect of different fertilizers on the productivity of these soils, it was observed that all the soils have low reserves of nitrogen for vegetation growth. This was shown clearly in the yield obtained for the treatment without fertilizers added both in the first harvest and especially in the second harvest of the first pot experiment. This study showed that all the soils responded to the nitrogen fertilizer dramatically. The yields were also increased with addition of P and K as well as the nitrogen fertilizer, but not by P+K alone. This shows importance of the earlier low results for available nitrogen in the soil, which were obtained in chapter 4 and also at the beginning of this experiment. Sykes et al.(1981) studied plant growth in soil containing chromium(III) and reported that chromium did not have a toxicity effect on plant growth. They also pointed out that in their experiment, nitrogen was the limiting factor for growth, not chromium. Soil 4 had lowest response to the fertilizer in the preliminary experiment, which raised the question about the possible toxicity of available Cr on the plant growth of this soil. There is also concern about the effects of physical problems of this soil on plant growth or effects of water logging on the soil microorganisms. Douglas and Crawford (1993) also found that the dry matter yield in soil with nitrogen applied, is lower in more compact soil conditions, compared with the yield obtained with the same rate of nitrogen application in soil with good physical conditions.

From the experiment in which different levels of the nitrogen fertilizer were applied to the soils, it was concluded that the application of 100 /50 /50 of N/P/K fertilizer can be a suitable rate for the site. Douglas and Crawford (1993) also obtained a lower rate of yield increase at addition of more than 100kgN/ ha. The nitrogen response curve of soil



sample 4 from the base of the east side of the slope was not the same as the other soils. Since the changes in the yield from this sampling point were not directly affected by available Cr in the two experiments the possibility of the effect being due to Cr only on plant growth was discounted (see Table 5.11).

There is also the possibility that the fertilizer nitrogen added to contaminated spoils may be less available to plants than the same amount of N added to agricultural soil due to toxicity to nitrogen transformations and also potential volatilization. How healthy is the nitrogen turnover process in these soils? Is it affected by toxicity? Is there any possibility of nitrogen loss happening in these soils?

Since nitrogen availability is more dependant on biological processes than physical or chemical processes ( Mengel and Kirkby, 1978) further experiments are necessary to look at the nitrogen turnover in these soils i.e. mineralization, nitrification and volatilization due to the high pH.

## CHAPTER SIX

### NITROGEN MINERALIZATION

#### 6.1 Introduction

From the pot experiments which were conducted to look at the effect of fertilizers on the productivity of these soils, it was observed that all soils had low levels of inorganic nitrogen ( $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ). This study also showed that the all soils responded well to the nitrogen fertilizer. The first pot experiment also showed that the nitrogen is a limiting factor for plant growth and when nitrogen was applied at higher rate then other factors become limiting, which might be P supply or physical conditions.

Since the change in the yield from the soils, especially soil 4, were not directly affected by available Cr in the two pot experiments, the possibility of the direct effect of Cr only on plant growth was discounted. However, the question was raised about how healthy is the nitrogen turnover process in these soils. The toxic effect of Cr on nitrogen transformation has been reported by a number of workers: Liang and Tabatabai (1977), Chang and Broadbent (1982), James and Bartlett (1984), Ueda et al. (1988), Ross, Sjogren and Bartlett (1981) and Wong and Trevors (1988).

Nitrogen mineralization is a very important process for the transformation of organic-N into inorganic form of nitrogen. Direct measurement of nitrogen mineralization provides a useful guideline for all aspects of nitrogen management including environmental impacts. Is the nitrogen transformation of these soils affected by Cr toxicity? Since the nitrogen mineralization is the decomposition of the organic nitrogen to inorganic nitrogen, the relation of the percentage of the organic matter to this process in these soils is also a matter of concern.

The pHs of these soils are also high. Is there any possibility of nitrogen loss due to ammonia volatilization in these soils because of high pH?

In order to look at the nitrogen turnover in these soils and possible toxicity effect of Cr on these processes, first it was decided to assess the nitrogen supply power i.e. nitrogen mineralization of the soils. That is because nitrogen mineralization is a valuable indicator for maintaining the plant growth in the soil. Incubation experiments can be carried out both in laboratory and field condition. Different parameters such as temperature, leaching, plant uptake or denitrification may change in the field incubation experiment. In order to simplify these circumstances, nitrogen dynamics and turnover in soil are often studied by incubation experiments under controlled and uniform condition of moisture and temperature. These experiments yield only net N transformation rates, calculated as the total change of inorganic-N during the incubation.

In order to determine the nitrogen mineralization rate of the soils from this site an incubation study was carried out. This experiment was done under aerobic conditions. An attempt was also made to look at possible volatilization of ammonia due to the high pH of these soils. Jar incubation was chosen to be used for nitrogen mineralization compared with leaching incubation because of ease of handling large number of samples and have more control some parameters like temperature. Moreover this method has previously been used successfully in different studies (Shah, 1988; Amin, 1995; Metwaly, 1999).

## **6.2 Materials and Methods**

### **6.2.1 Soil Sampling and Preparation**

Six soil samples were taken from the six sampling points of the site. The descriptions of these sampling points are given in section 5.2.1.5. These sampling points are shown in Figure 4.11. Soil samples were taken from the 0-15cm depth. The surface vegetation, dead leaves and the branches were removed from the topsoil and all soil



samples were put in plastic bags, labeled and carried to the laboratory. Each soil was then spread on the clean plastic sheet, the large aggregates were partly broken down. Each soil sample was mixed thoroughly and was allowed to partially dry at room temperature. Then the soils were passed through a 4mm sieve.

### **6.2.2 Analysis of Soil Samples**

The moisture content of soils was determined with the method described in section 2.1.5. The moisture content of soils at  $-0.5$  bar was measured as described in section 2.1.7. The soil samples were extracted for available inorganic nitrogen (ammonium-N, nitrate-N and nitrite-N), available phosphorus, available potassium and magnesium as described in section 2.2.1, 2.2.3 and 2.2.4 respectively. The extracts were analyzed for ammonium-N, nitrite-N and nitrate-N with the methods described in section 2.4.3, 2.4.5 and 2.4.6 respectively. The available P was measured by method described in section 2.4.7. The available K and available Mg were measured using the methods described in sections 2.4.9 and 2.4.2. The water soluble Cr was extracted by method described in section 2.2.2 and measured with the method described in section 2.4.11. The  $K_2SO_4$  extractable Cr was also measured by AAS with the method described in section 2.4.1. The pH, Loss On Ignition and percentage of organic matter of soils were measured using the methods described in sections 2.1.3, 2.1.8 and 2.1.9 respectively.

### **6.2.3 Preparation of 0.1M Hydrochloric Acid Solution**

The amount of 8.5ml of concentrated hydrochloric acid was mixed with deionized water. It was then made up to 1 litre with deionized water.

### **6.2.4 Incubation Procedure**

Soil samples were partly dried until below the  $-0.5$  bar moisture content. Their moisture content was then measured. An amount of equivalent of 100g oven dried soil from this partly dried (below  $-0.5$  bar) fresh soil was weighed into a 1.5 litre Kilner jar.

An appropriate amount of water was added to each soil sample to bring the moisture content to -0.5 bar percentage using a Pasteur pipette. The experiment was carried out with four replicates of each soil.

15ml of 0.1M hydrochloric acid was pipetted into a 100ml plastic bottle, which was then put on top of each soil in the Kilner jar. The Kilner jars were then sealed in order to prevent loss of ammonia gas produced. The red rubber sealing rings were replaced with parafilm in order to prevent inhibition of the nitrifying bacteria. The jars were then placed in an incubator at 20°C. The jars were large enough to contain O<sub>2</sub> for the incubation for a week until the next sub-sampling time. Blank incubations in which glass bottles contained only 0.1M HCl with no soil were also carried out in order to correct for possible ammonia gas adsorption from the air. The incubation was carried out for a period of 17 weeks.

Sub samples of each bottle were taken at several times at one week intervals and extracted with 0.5M K<sub>2</sub>SO<sub>4</sub> to be analyzed for inorganic nitrogen content (NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N). At each sampling time, the plastic bottles containing 0.1M HCl were taken off and their volumes were made to 25ml with 0.1M HCl in 25ml volumetric flasks. Then they were replaced with new plastic bottles containing 15ml fresh 0.1M HCl. Fresh air was introduced to the glass bottles for 10-12 minutes before sealing off the jars in every sub-sampling time. The 0.5M K<sub>2</sub>SO<sub>4</sub> extract solutions were analyzed for their content of inorganic -N (NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N) as described in section 2.4.3, 2.4.5 and 2.4.6 respectively. The acid solutions were also analyzed for their content of NH<sub>4</sub><sup>+</sup>-N by method described in 2.4.3. The pH of soils also was measured at the end of experiment as described in section 2.1.3. The available P and K were extracted at the end of the experiment with the methods described in section 2.2.3 and 2.2.4 and measured using methods described in section 2.4.7, 2.4.9. The amount of Cr in the K<sub>2</sub>SO<sub>4</sub> extracts from weeks 0, 1, 4, 13 and 17 was measured by AAS as described in section 2.4.1. The amount of Cr in water extracts of the soils at the week 0 and 17 was measured by AAS as described in section 2.4.1.



### 6.3 Results and Discussion

The results of the pH, percentage of organic matter, loss on ignition (LOI), total-N, total-Cr, initial soil extractable inorganic-N ( $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ), extractable phosphorus, water extractable Cr and  $\text{K}_2\text{SO}_4$  extractable Cr are shown in Table 6.1.

As it can be observed the pH of all soils are uniformly high due to the presence of carbonate rich waste material. The percentages of loss on ignition in these soils are much higher than the percentage of organic matter. This was because the determination of the organic matter by LOI (Loss On Ignition) has the potential of overestimation in soils containing large amounts of carbonate, due to the breakdown of carbonates. The values of the total nitrogen in these soils are very low and the values of total chromium are very high. The initial extractable nitrogen in soils showed there was some extractable ammonium-N in the soils, which had not converted to nitrate. Extractable phosphorus is variable throughout the site with higher levels in soils 1, 2, 5 and 6 and lower levels in soils 3 and 4. The soil  $\text{K}_2\text{SO}_4$  extractable Cr is higher than water extractable Cr and this is because salt solution can extract more ions from exchange sites in the soils.



Soil NO	pH	OM %	% LOI	Total N $\psi$ %	Total Cr $\psi$ mg/kg	Initial extractable-N mg/kg			Extractable phosphate-P mg/kg	Extractable Cr mg/kg	
						NH <sub>4</sub> <sup>+</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	NO <sub>3</sub> <sup>-</sup> -N		Water	K <sub>2</sub> SO <sub>4</sub>
1	8.38	7.99	15.54	0.36	2070	4.32	ND *	1.12	33.9	0.93	1.47
2	8.32	7.78	13.44	0.36	2270	7.55	ND	ND $\theta$	13.9	1.47	1.60
3	8.64	5.97	11.29	0.09	9230	0.81	ND	ND	9.2	1.80	2.07
4	8.39	4.40	9.30	0.08	15200	1.36	ND	ND	3.3	2.87	5.27
5	8.57	6.42	11.78	0.21	13400	1.71	ND	ND	34.8	1.53	2.07
6	8.46	7.40	12.78	0.19	1380	1.06	ND	ND	18.0	1.20	1.27

Table 6.1: The chemical properties of the soils used for the mineralization incubation.

\*ND: Non detectable < 0.005 mg/l extract solution or 0.1 mg/kg soil.

$\theta$  ND: Non detectable < 0.025 mg/l extract solution or 0.5 mg/kg soil.

$\psi$ : Data from soil survey results.

In the mineralization study the concentration of NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N were measured by extraction at intervals. The sum of NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N was calculated as total inorganic nitrogen. The changes in ammonium-N, Nitrite-N, nitrate-N and total-N for incubated soils 1–6 are illustrated in Figures 6.1–6.6. An attempt was made to standardize the graph axis to allow easy comparison. Soils 1 and 2 were shown with the same axis due to their similar higher inorganic nitrogen levels. Soils 3 and 4 were shown with the same axis due to lack of mineralization in these two soils. And finally soils 5 and 6 were shown with the same axis due to their similar lower inorganic nitrogen production.

As is shown in the figures the initial nitrogen decreased in all soils in the first week. After this decrease there was a lag period (no change of inorganic-N) for approximately 2–3 weeks followed by an increase in nitrate and total-N in four soils 1, 2, 5 and 6 but no change appeared in soils 3 and 4. There was no detectable nitrite-N in any soils. The acid solution used for trapping ammonia gas showed no detectable volatile NH<sub>3</sub>. All soils showed a low level of ammonium-N during the incubation period.

Consequently the total inorganic–N is influenced by the concentration of  $\text{NO}_3^-$ –N. This also accounts for the non detectable ammonia gas volatilization from these soils.



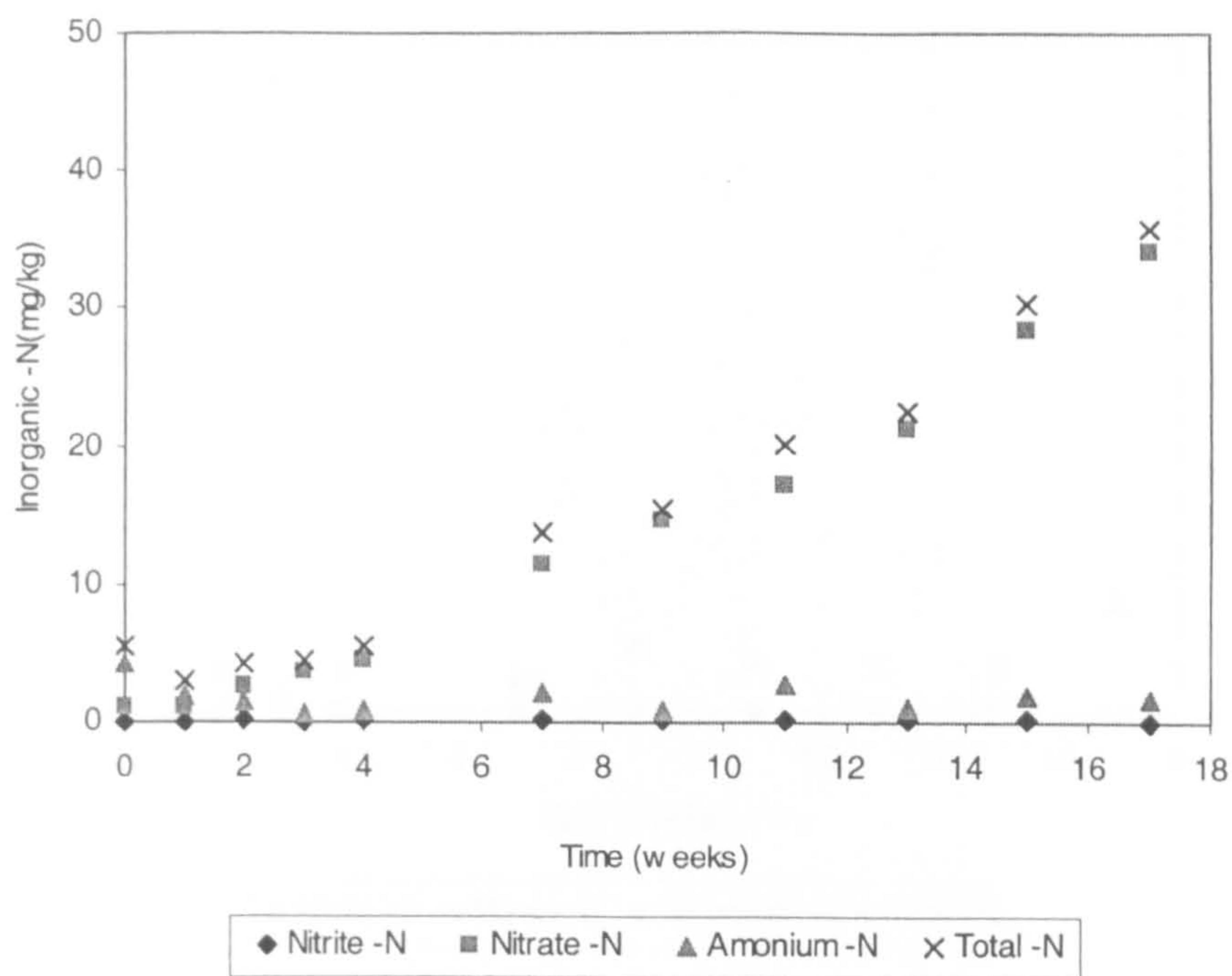


Figure 6.1: The changes in inorganic-N during the incubation of soil 1 (mean of 4 replicates).

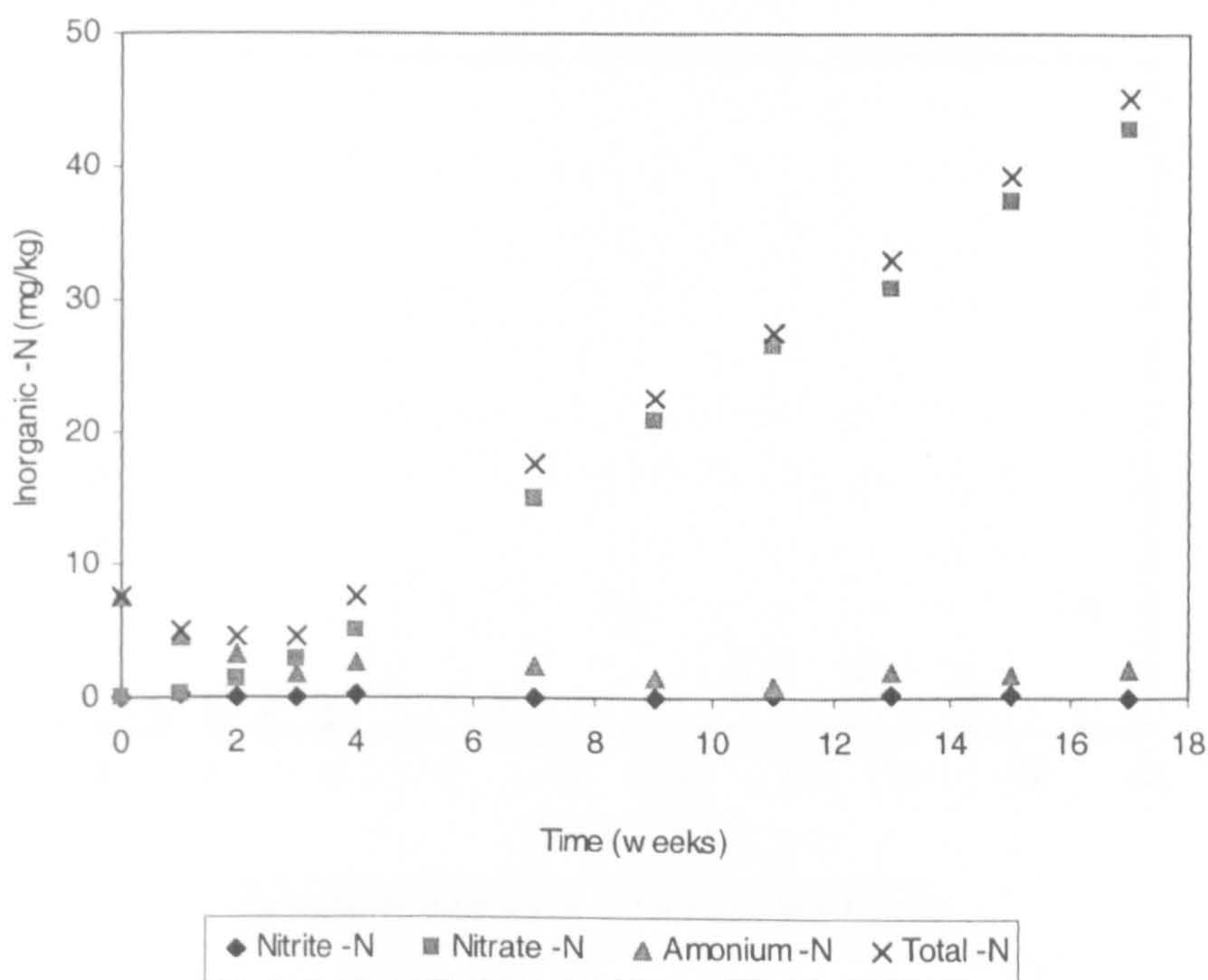


Figure 6.2: The changes in inorganic-N during the incubation of soil 2 (mean of 4 replicates).

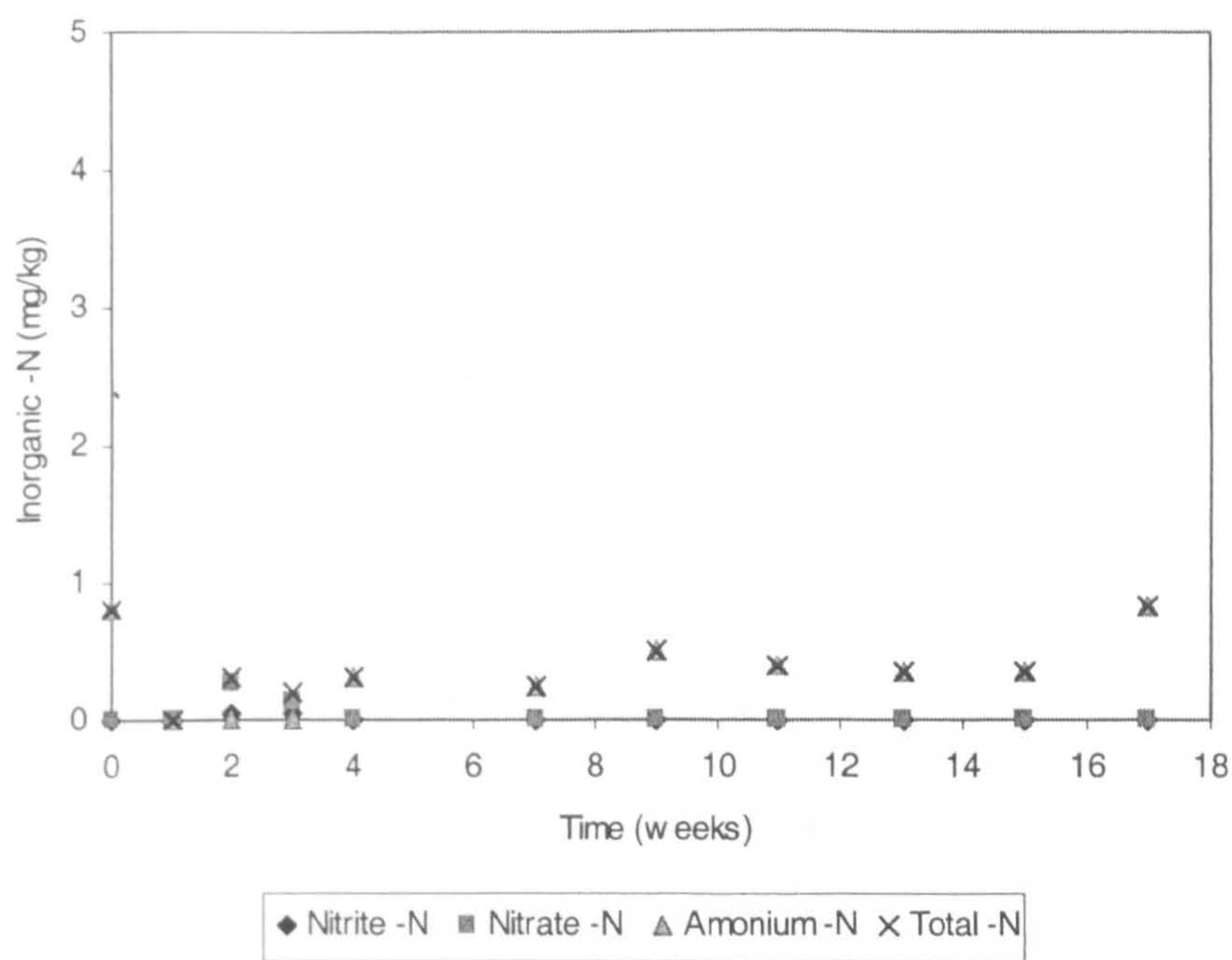


Figure 6.3: The changes in inorganic-N during the incubation of soil 3 (mean of 4 replicates).

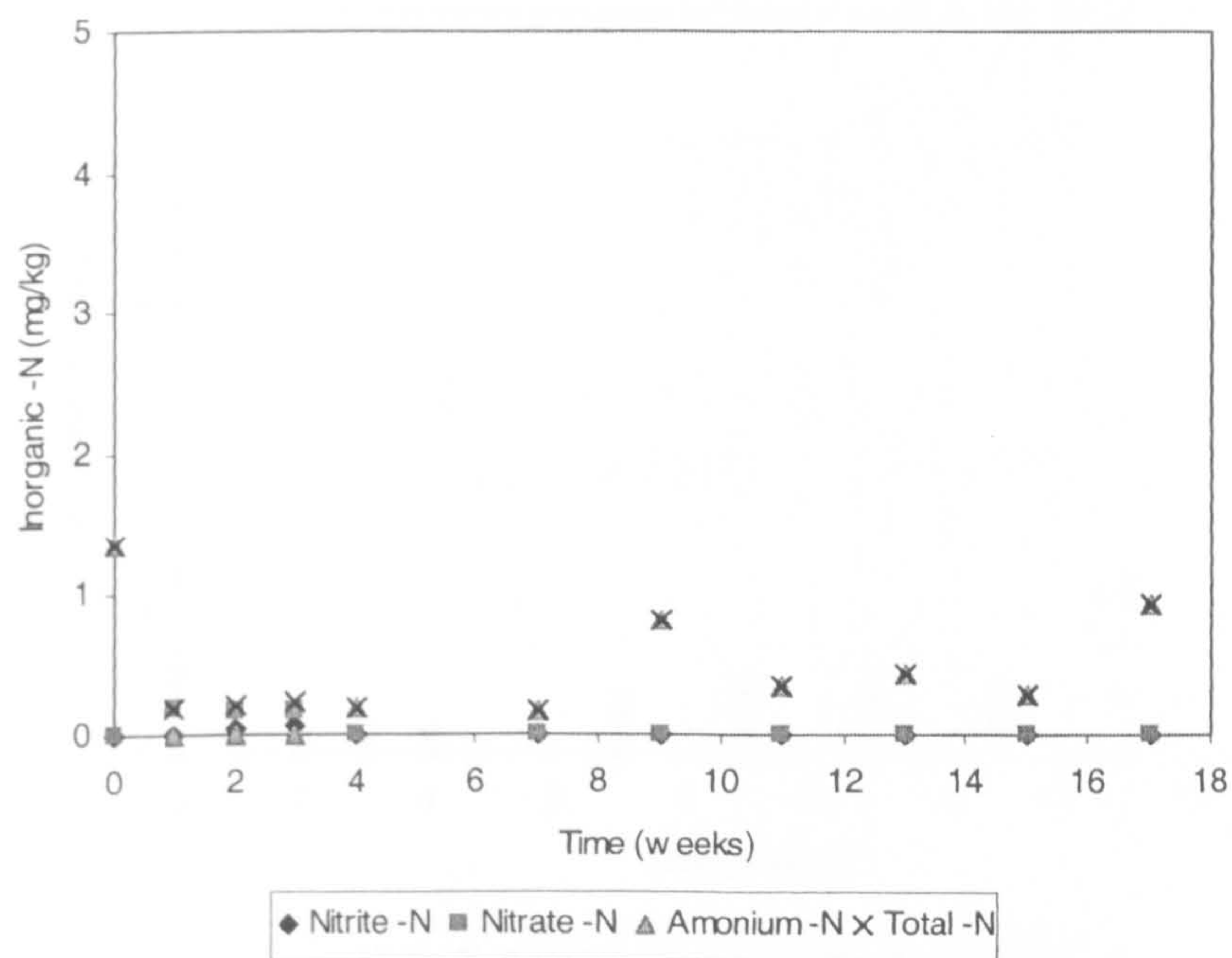


Figure 6.4: The changes in inorganic-N during the incubation of soil 4 (mean of 4 replicates).



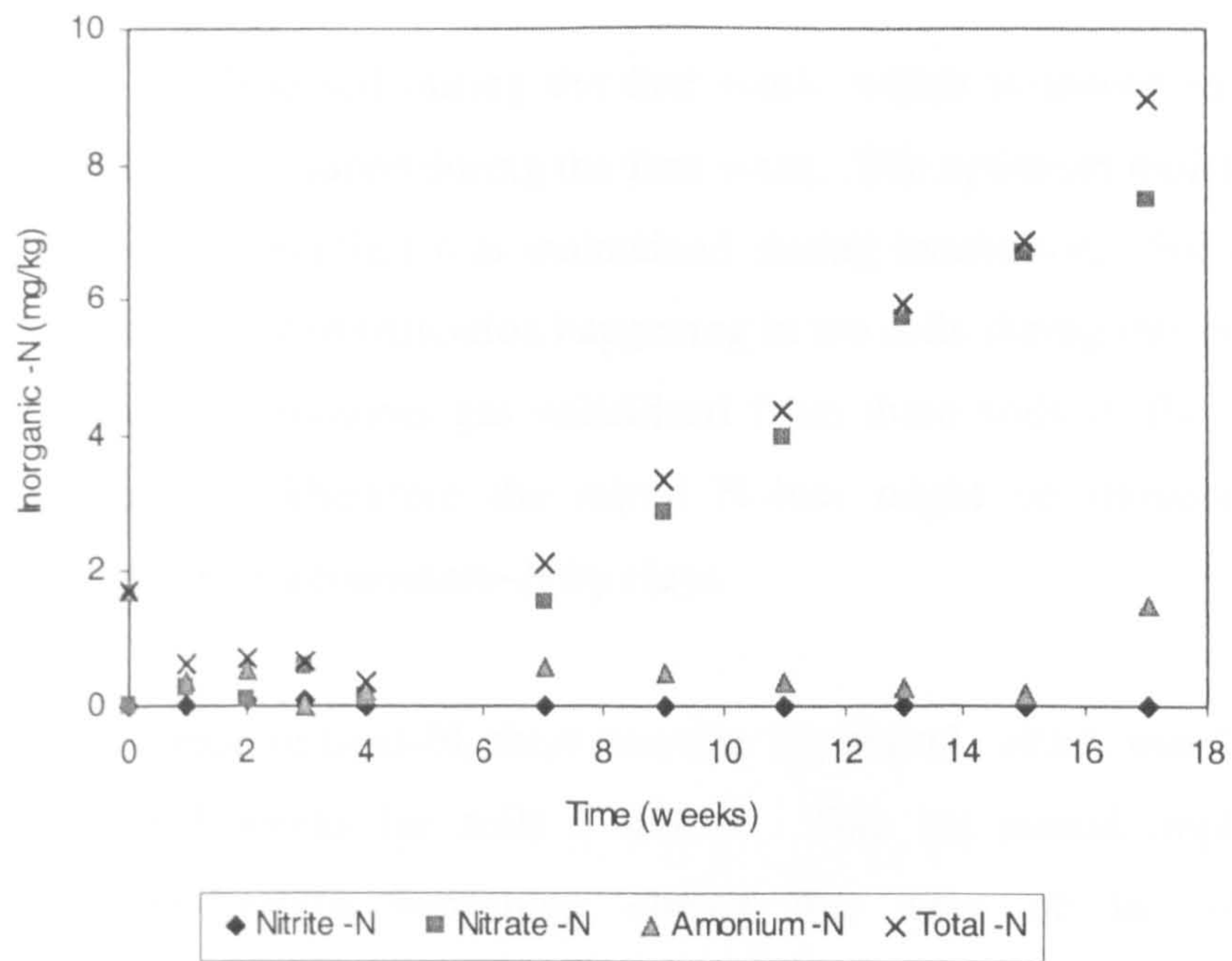


Figure 6.5: The changes in inorganic–N during the incubation of soil 5 (mean of 4 replicates).

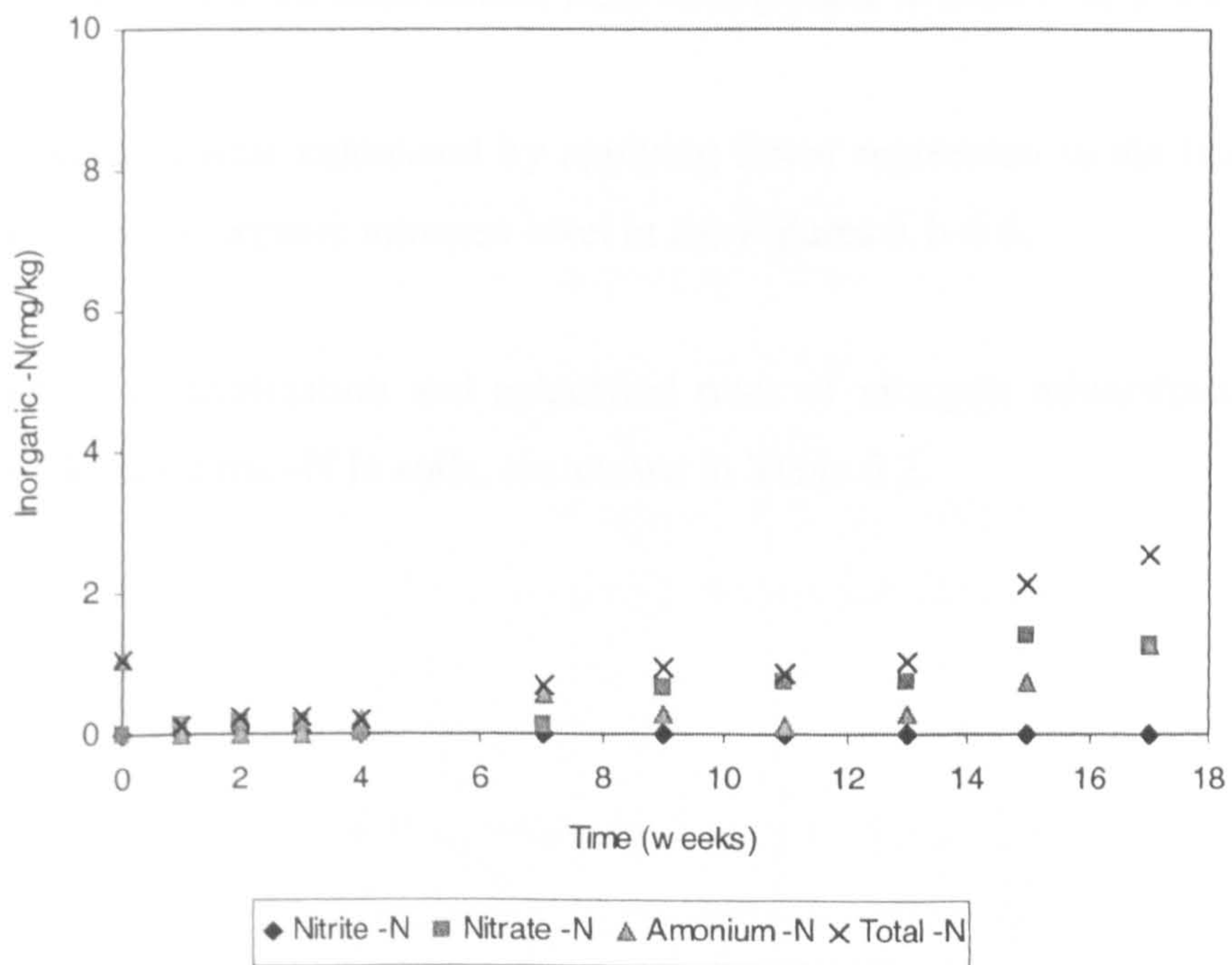


Figure 6.6: The changes in inorganic–N during the incubation of soil 6 (mean of 4 replicates).

The level of total-N decreased during the first week, which is shown in Table 6.2. There was no nitrate-N produced during the first week. The optimum moisture content (-0.5 bar soil moisture potential) was maintained during incubation. For that reason there should have been no denitrification happening in the soils during this period. Also there was no detectable ammonia gas volatilized from these soils in this experiment (data are not shown). Therefore the initial N-loss might be immobilization of inorganic-N or fixation of ammonium-N by clays.

After the initial decrease in total-N, there was the lag period, which was 2 weeks for soils 1 and 2, and 3 weeks for soils 5 and 6. This lag period implies no net mineralization-immobilization happening during this time or in other words mineralization = consumption. In this period presumably any ammonium produced was used by bacteria for built up, growth and general increase.

After the lag period the concentration of  $\text{NO}_3^-$ -N and total-N increased which showed that both mineralization and nitrification were taking place in soils 1, 2, 5 and 6.

Mineralization rates were calculated by applying linear regression to the linear part of the increase in total inorganic nitrogen level in the Figures 6.1–6.6.

The duration of mineralization and calculated rates of nitrogen mineralization in mg N/kg soil/week, inorganic-N in soils, are shown in Table 6.2.



Soil NO.	Total -N decrease in the first week (mg/kg)	Mineralization weeks	Mineralization rate (mg/kg/week)	
1	2.42	3 –17	2.22	HS*
2	2.64	3 –17	2.83	HS
3	0.81	–	0.03	NS*
4	1.16	–	0.03	NS
5	1.10	4 – 17	0.64	HS
6	0.93	4 –17	0.17	VS*

Table 6.2: The slope (rate) of changes in total–N and amount of nitrogen decrease in the first week in N–mineralization incubation.

Levels of mineralization rates are significantly different from 0.

\*HS: Highly Significant p<0.001.

\*VS: Very Significant p<0.01.

S: Significant p<0.05.

\*NS: Non Significant p>0.05.

Soils 1 and 2 showed the highest linear rates of nitrogen mineralization. In soil 5 and 6 the first week decrease in total–N was followed by a three weeks lag period. Then soils 5 and 6 showed linear rates of nitrogen mineralization but the rate and final concentration of produced inorganic–N was less than soils 1 and 2. Soils 3 and 4 showed non significant slope of the regression of N-mineralization rates from the 0 during the incubation period. This means no mineralization was happening in these soils.

The capacity of soil to mineralize nitrogen is usually determined by measuring the amount of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N released in the specific period of time under controlled conditions. The rate of release of inorganic–N reflects the potential availability of NH<sub>4</sub><sup>+</sup>-N to microorganisms and plants.



Different researchers have obtained different rates for nitrogen mineralization. They used different temperature and duration for incubation based on the soil variation. Some of these rates and conditions they obtained are summarized in Table 6.3.

Author	Temperature °C	Time of incubation	Mineralization Rate mgN/kgsoil/week	Soil type
Tabatabai and Al-Khafaji, 1980	20-35°C	26 weeks	2.9 at 20°C	
Flowers and Arnold 1983	15°C 30°C	25 weeks	1.3 – 2.6 at 15°C 4.9 – 7.2 at 30°C	Arable
Addiscott 1983	5, 10, 15, 20 and 25	20 weeks	0.938 at 20°C 2.233 at 25°C 1.827 at 20°C	Grass Farmyard manure Arable
Wiseman and Zibilske 1988		12 weeks	5.92	Non amended soil
Singha et al. 1998	Not mentioned	16	3.7	Non amended soil
Aggangan et al. 1999	20°C	29 weeks	0.39 native forest 3.09 pasture soil	Agroforestly
Abbasi et al. 2001	20°C	7 weeks	6.51	Grassland

Table 6.3: Nitrogen mineralization rates obtained by different researchers.

Variation in mineralization rates may be attributed to different labile organic nitrogen compounds or a variation in level of microbial biomass and activity.

As it can be observed the rate of mineralization in soils 1 and 2 is comparable with some arable soils and less than some others while the rates for soils 5 and 6 showed that despite existence of some mineralization they are much less than the natural soils. Soils 3 and 4 obviously showed no mineralization with very low level ammonium-N after 8 weeks.

Overall these soils mainly showed a low level of initial inorganic nitrogen in soil, low conversion of ammonium-N to nitrate-N in natural (condition) system. From the results of the field soils it is shown that there was some extractable ammonium-N in the soils, which did not, convert to nitrate. This shows a very low nitrogen turnover in the soils.

The correlation analysis was applied by Minitab to see the possible significant effects from different parameters on soil nitrogen mineralization.

The available Cr is highest in the soil 4 but the values of available Cr in other soils are almost the same. Therefore the differences in mineralization can not be attributed to available Cr in these soils. There was no significant correlation between any extractable Cr, neither water extractable, nor  $K_2SO_4$  extractable Cr and mineralization rates calculated for these soils ( $p>0.05$ ).

Wiseman and Zibilske (1988) studied the effect of amendment of Cr containing sludge on soil N-mineralization. They incubated a fine sandy loam for 84 days at 24-26°C. They obtained 5.92 mgN/ kg soil/ week for un-amended control soil. They did not observe any adverse effect of accumulation of Cr on mineralization of the soil when different kinds of sludge were amended to the soils. The nitrogen mineralization rates were 13.75 and 13.67 mgN/ kg soil/ week for domestic sludge and municipal sludge amended soils respectively. Even when the total Cr of the soil was 700mg Cr/kg soil after two application of municipal sludge the N-mineralization increased to 20.58 mgN/ kg soil/ week. They concluded that the soil microflora could tolerate higher levels of Cr from organic source than would be predicted from the soil incubated studies using



soluble Cr salts. Liang and Tabatabai (1977) studied the effect of different heavy metals solution on nitrogen mineralization. They incubated 10g soil with 3ml of solution containing 50  $\mu$ mole of Cr(III) (7.8mg) which correspond to 780mg/kg. They observed that the Cr(III) had inhibitory effect on this process. They mentioned that the accumulation of heavy metals could lead to reduction in the amount of plant available nitrogen derived from soil organic matter and the degree of effectiveness is different in different soils.

The mineralization rates in soils 1 and 2 are highest. They have higher organic matter content while other soils like soil 6, which have very low mineralization rate, also have higher organic matter. Therefore the mineralization rates in these soils cannot be regarded as a result of the concentration of the soil organic matter. The correlation analysis of the results also showed that the nitrogen mineralization rates in these soils are not correlated to the percentage of organic matter in the soils ( $p>0.05$ ). In the study, which was done by Roşs et al. (1981) on the effect of Cr on soil microorganisms, they mentioned that the Cr might have formed cross-linkages between organic compounds in the soils rendering them unavailable to microbial attack.

Since the pHs of all soils are uniformly high, the changes in mineralization rates cannot be because of changes in the soil pH. The statistic correlation analysis also showed there was no correlation between the pH of soils at the beginning of incubation and obtained nitrogen mineralization rates ( $p>0.05$ ). The pH of the soils showed a slightly decrease after the incubation period (data are not shown).

The calculated C:N values for these soils are shown in Table 6.4. As it can be observed the soils 3 and 4 have the highest C:N values compared with the other soils. Correlation analysis of the results also showed that there is a significant correlation between C:N values in the soils and obtained nitrogen mineralization rates. The nature of organic matter can affect the mineralization behavior in the soils. Some organic matter contain higher nitrogen than the others and some readily decomposable. Whether or not there is nitrogen surplus to the needs of the micro-organisms, so that net mineralization can



occur, depends on the C:N ratio of the substrate and on the properties of the decomposer organisms.

In the study which was done by Janssen (1996) on N mineralization of various organic material he observed that the fraction of mineralized-N is linearly related to substrate C:N for decomposable material. Generally, when the C:N ratio is  $> 25$ , net immobilization occurs, whereas at ratios  $< 25$  net mineralization is likely (White, 1997). Trinsoutrot et al. (2000) studied N-mineralization of soil with different crop residues. They set up an incubation for the study of the N-mineralization of fresh soils for the 168 days at  $15^{\circ}\text{C}$ . They found that only the soil residues with a C:N ratio  $< 24$  induced a surplus of mineral-N and showed net mineralization. They found a range of mineralization and immobilization based on C:N ratio of the soils ( $+50\text{gN /kgC}$  for alfalfa to  $-28\text{gN/kgC}$  for maize straw). Overall the C:N ratio is a reliable factor to predict the net effect of plant residue on soil mineral-N dynamics. Abbasi et al. (2001) mentioned that the rapid release of inorganic nitrogen through mineralization from the organic material with C:N ratio of  $< 20$  is well established.

Zaman et al., 1999 studied the mineralization of soils and their relationship with microbial biomass. They concluded that the higher nitrogen mineralization rates can be due to the presence of readily mineralizable organic substances with a low C:N ratio which stimulate microbial activity. They pointed out that the low and stable nitrogen mineralization probably might have resulted from a more stable organic N compound.

The available phosphorus in soils before and after incubation are shown in Table 6.4. Since the phosphorus values had not changed after mineralization incubation it can be concluded that net phosphorus mineralization had not occurred in these soils.

Soil NO.	C/N	Available P before mg/kg	Available P after mg/kg
1	13.01	33.9	33.6
2	12.41	13.9	13.6
3	37.16	9.2	9.1
4	34.09	3.3	4.0
5	18.04	34.8	33.6
6	22.94	18.0	16.5

Table 6.4: The C:N values and the concentration of soil available phosphorus before and after mineralization incubation (mg/kg).

The available Cr at the beginning and the end of incubation are shown in Table 6.5. Despite a minor increase, which can be observed in soil extractable Cr at the end of the experiment, it can be seen that no major systematic changes of available Cr happened during incubation.

Soil NO	Water Extractable Cr		K <sub>2</sub> SO <sub>4</sub> Extractable Cr				
	Week 0	Week 17	Week 0	Week 1	Week 4	Week 8	Week 17
1	0.93	1.15	1.47	1.55	1.55	1.60	1.60
2	1.47	1.85	1.60	1.80	1.85	1.90	1.95
3	1.80	2.20	2.07	2.00	2.25	2.40	2.75
4	2.87	3.70	5.27	5.20	5.30	5.10	5.15
5	1.53	2.05	2.07	2.05	1.95	2.10	2.15
6	1.20	1.70	1.27	1.35	1.20	1.70	1.95

Table 6.5: The water extractable Cr at the end and K<sub>2</sub>SO<sub>4</sub> extractable Cr in different weeks of the mineralization incubation (mg/kg).



## 6.4 Conclusion

From the results obtained for nitrogen mineralization for these soils it can be concluded that the system shows low nitrogen turnover. Some soils showed slightly better nitrogen mineralization than others.

Mineralization in soils 1 and 2 is comparable with some arable soils (Tabatabai and Al-Khafaji, 1980) while the rates for soils 5 and 6 showed that, despite existence of some mineralization, they are much less than the natural soils. Soils 3 and 4 obviously showed no mineralization. There is a significant correlation between C:N values in the soils of this site and nitrogen mineralization rates obtained. The low and stable nitrogen mineralization of these soils presumably might have resulted from a more stable organic N compound and high C:N ratio compared with readily mineralizable organic substances with a low C:N ratio which stimulate microbial activity. This is in agreement with findings of different studies. Trinsoutrot et al. (2000) also found that any residue with C:N ratio  $< 24$  indicates a surplus of mineral nitrogen and showed nitrogen mineralization. Zaman et al. (1999) studied the mineralization of soil treated with dairy shed effluent (DSE) or  $\text{NH}_4^+$  fertilizer in an incubation study at  $20^\circ\text{C}$ . They found that mineralization rate increased with addition of (DSE). They attributed the higher mineralization rate to the presence of readily mineralizable organic substances with low C:N ratio and stimulation of soil microbial activities. Janssen (1996) and Wong (1999) also pointed out that the C:N value of the soil is a good reliable factor to predict the effect of soil and crop residue on soil mineral nitrogen dynamic.

The presence of nitrate-N in some of these soils and absence in others indicates the need to investigate the nitrification process in these soils. How is this part of nitrogen transformation in these soils? Is it affected by toxicity?

In order to investigate the occurrence of this phenomenon and the probable effects of toxicity of Cr on nitrification it was decided to carry out a nitrification incubation and nitrogen balance of these soils.



# CHAPTER SEVEN

## NITRIFICATION

### 7.1 Introduction

From the previous studies with the pot experiment it was observed that the soils had low levels of inorganic nitrogen ( $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ) and they responded well to the nitrogen fertilizer which shows nitrogen is the limiting factor. Since the change in the grass yield was not directly affected by available Cr it was decided to look at the nitrogen transformation in these soils and possible toxicity of Cr on these processes.

In the nitrogen mineralization incubation and volatilization tests which were carried out for 17 weeks to assess the nitrogen supply power of these soils it was observed that some soils showed mineralization and nitrate-N was the product of mineralization, while others did not reveal any mineralization during this period.

The presence of nitrate-N in some of these soils and absence in others indicates the need to investigate the nitrification process of added ammonium-N in these soils. How is this part of nitrogen transformation in these soils? Is it affected by toxicity?

The toxicity of heavy metal and Cr on nitrification was reported in a number of works [Liang and Tabatabai (1978), James and Bartlett (1984) and Kostov and Van Cleemput (2001)].

There are reports from some studies that the nitrification process is more sensitive than mineralization process to the changes in soil condition and excess of heavy metals (Sierra et al., 2001). They expressed this idea because a specific group of microorganisms is responsible for nitrification compared with a number of groups of microorganisms involved in mineralization. Therefore when toxicity occurs in the soil, the nitrification process might be damaged more than mineralization.

In order to investigate the occurrence of nitrification of added ammonium-N and the probable effects of toxicity of Cr on nitrification it was decided to carry out a nitrification incubation and nitrogen balance of these soils.

During this first incubation for nitrification, the amount of total inorganic nitrogen decreased in the first few days. This decrease was different with different soils. The pH of soils are high (8-9) due to the presence of carbonate rich waste material. This may have affected the added  $\text{NH}_4^+$ -N and changed it to ammonia gas and caused volatilization. The decrease of the inorganic-N could also have been caused through immobilization by microorganisms.

In order to look at the probable existence of volatilization, another nitrification incubation was carried out. This second incubation was short-term because the maximum decrease in total inorganic-N was observed in the first 10 days of the first nitrification incubation.

Incubation experiments can be carried out both in laboratory and field conditions. Different parameters such as temperature, leaching, plant uptake or denitrification may change in the field incubation experiment. In order to simplify these circumstances, nitrogen dynamics and turnover in soil are often studied by incubation experiments under controlled and uniform condition of moisture and temperature. These experiments yield only net N transformation rates, calculated as the total change of inorganic-N during the incubation.

In order to determine the nitrification rate of added ammonium in soils from this site an incubation study was carried out. Jar incubation was chosen to be used for nitrification compared with leaching incubation because of ease of handling large number of samples and have more control some parameters like temperature and volatilization. Moreover this method has previously been used successfully in different studies (Shah, 1988; Amin, 1995; Metwaly, 1999).



## **7.2 Materials and Methods**

### **7.2.1 Soil samples**

Six soil samples were taken from 6 sampling points of the site. The descriptions of sampling points were given in section 5.2.1.5.

### **7.2.2 Ammonium-N Solution (2000mg/l: $\text{NH}_4^+$ -N/l)**

Ammonium sulphate was dried in oven for one hour. Then an amount of 9.1429g ammonium sulphate ( $\text{NH}_4$ )<sub>2</sub>SO<sub>4</sub> was weighed and dissolved in deionized water. The volume of the solution was made up to one litre with deionized water.

### **7.2.3 0.1M Hydrochloric Acid Solution**

8.5ml of concentrated hydrochloric acid was mixed with deionized water. It was then made up to 1 litre with deionized water.

### **7.2.4 Analysis of Soil Samples**

The moisture content of soils was determined with the method described in section 2.1.5, and the moisture content of soils at -0.5 bar was also measured as described in section 2.1.7. The soil samples were extracted for available inorganic nitrogen (ammonium-N, nitrate-N and nitrite-N) as described in section 2.2.1. The extracts were analyzed for ammonium-N, nitrite-N and nitrate-N with the methods described in section 2.4.3, 2.4.5 and 2.4.6 respectively. The water soluble Cr was extracted by method described in section 2.2.2 and measured with the method described in section 2.4.1. The pH and percentage of organic matter of soils were measured using the methods described in sections 2.1.3 and 2.1.9 respectively.

## **7.2.5 Procedures**

### **7.2.5.1 First Incubation Method**

Soil samples were partly dried until below the  $-0.5$  bar moisture content. Their moisture content was then measured. An amount equivalent to 100g oven dried soil from this partly dried (below  $-0.5$  bar) fresh soil was weighed into a 500ml glass bottle. Each sample was treated with 100mgN/kgsoil (mg/kg: N/soil) by adding 5ml of ammonium sulphate solution containing 2000mg  $\text{NH}_4^+\text{-N/l}$ . An appropriate amount of water was needed to bring the moisture content of each soil sample to  $-0.5$  bar percentage using a Pasteur pipette. Each bottle content of soil sample was mixed thoroughly using a spatula. The experiment was carried out with four replicates of each soil.

The bottles were placed in large plastic bins. The bins were lined with damp filter paper with a thin layer of deionized water in the base. This was done in order to maintain a humid atmosphere to prevent change in moisture content of the soils. Then the lids of bins were put on to prevent water loss. The bins were placed in an incubator at  $20^\circ\text{C}$ . The bins were large enough to contain  $\text{O}_2$  for incubation and aeration for bacteria for at least one week. Four blanks were also carried out.

Sub samples of each bottle were taken at several times at 2-3 day intervals. They were extracted with  $\text{K}_2\text{SO}_4$  using method 2.2.1. The glass bottles were left open for 10–15 minutes to allow aeration with fresh air at the end of each interval. Care was also taken to adjust loss of moisture weight of the soil during interval with water and mixing them thoroughly before taking next sub-samples at the next sampling time.

The  $\text{NH}_4^+\text{-N}$  was measured in the extracts using method described in section 2.4.3 with appropriate standard solution of 5mg/l. The  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$  in these extracts also were measured using methods described in sections 2.4.5 and 2.4.6 respectively with 1mg/l standard solution for nitrite-N and 5mg/l solution for nitrate-N. The values were used to calculate the nitrification rates. The soil samples were incubated until the conversion (transformation) of ammonium to



nitrate was completed. The pH was also measured at the end of experiment to observe if there is any change in soil pH.

#### **7.2.5.2 Second Incubation Method**

The experiment was carried out with four replications of each soil. The amount equivalent to 100g oven dried soil from partly dried fresh soil was weighed into a Kilner jar. The sample was treated with 100mg/kg:  $\text{NH}_4^+\text{-N}$  /soil by adding 5ml of ammonium sulphate solution containing 2000mg/l of N. For each soil sample an appropriate amount of water was needed to bring the moisture content to -0.5 bar percentage using a Pasteur pipette.

35ml of 0.1M hydrochloric acid was pipetted into a 100ml plastic bottle, which was then put on top of each soil in the Kilner jar. The Kilner jars were then sealed in order to prevent loss of ammonia gas produced. The red rubber sealing rings were replaced with parafilm in order to prevent inhibition of the nitrifying bacteria. The jars were then placed in an incubator at 20°C. The jars were large enough to contain  $\text{O}_2$  for aeration of bacteria for the short-term incubation of 10days period. Blank incubations in which glass bottles contained only 0.1M HCl with no soil were also carried out in order to correct for possible ammonia gas adsorption from the air.

After 10 days glass bottles were opened. The plastic bottles containing hydrochloric acid were taken off and their lids were put back on. They then kept in the cold room at 2°C temperature to be analyzed at appropriate time. After 10 days sub samples of each bottle were taken, extracted and the amounts of extractable ammonium-N, nitrite-N and nitrate-N were measured. The volume of hydrochloric acid was made up to 50ml with 0.1M hydrochloric acid in 50ml volumetric flasks. They were then analyzed for ammonium-N. The pH of the soils were also measured at the end of the experiment.

## 7.3 Results and Discussion

### 7.3.1 First Incubation

Freshly collected soil samples were used and care was taken to ensure that soils received only the minimal amount of air drying so as to minimize any reduction in the nitrification population, as suggested by Flowers and O'Callaghan (1983). The pH values from the samples before and after incubation, percentage of organic matter, initial inorganic nitrogen and available Cr before and after nitrification incubation are shown in Table 7.1.

Soil NO	pH		OM %	Initial -N mg/kg			Water available Cr AA mg/kg	
	Before	After		NH <sub>4</sub> <sup>+</sup> -N	NO <sub>2</sub> <sup>-</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	Before	After
1	8.08	7.71	7.93	2.03	ND *	7.32	0.65	0.83
2	8.13	7.87	7.62	2.03	ND	4.07	1.13	1.39
3	8.26	8.04	5.73	0.43	ND	0.08	1.32	1.66
4	8.46	8.29	3.93	1.90	ND	0.33	2.27	2.89
5	8.32	8.00	6.44	0.73	ND	0.36	1.12	1.53
6	8.21	8.12	7.36	1.48	ND	0.71	0.90	1.84

Table 7.1: Some chemical properties of soils used for nitrification incubation.

\*ND: Non detectable < 0.005 mg/l extract solution or 0.1 mg/kg soil.

As it can be observed the pH of all soils are uniformly high due to the presence of carbonate rich waste material. The initial extractable nitrogen in the soils showed there was some extractable ammonium-N in the soils, which was partly converted to nitrate-N in some soils.

The inorganic nitrogen at the time zero in soils was calculated by adding the initial inorganic-N (NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N) to added 100mg/kg: NH<sub>4</sub><sup>+</sup>-N /soil. The changes in ammonium-N, Nitrite-N, nitrate-N and total inorganic-N for the incubated soils 1-6 are illustrated in Figures 7.1-7.6 respectively. Each figure demonstrates the mean of 4 replicates used in this study.



It can be observed in the figures that all soils show nitrification of the added ammonium. The nitrate-N increased slowly in the first few days, followed by an increase with higher slope in soils 1 and 2 at the later stages. The increase of nitrate-N in soils 3, 4, 5 and 6 started after a delay. This delay or lag period was between 4-6 days in the different soils. A loss of total inorganic-N was observed in all soils in the first few days of the incubation. This was followed by a slight rise until the end of the incubation period. The length of time for decrease of total inorganic-N varied in different soils. There was a linear phase of  $\text{NO}_3^-$ -N increase which corresponds to the linear phase of the  $\text{NH}_4^+$ -N decrease. There was an accumulation of  $\text{NO}_2^-$ -N in all soils, which varied in the different soils.

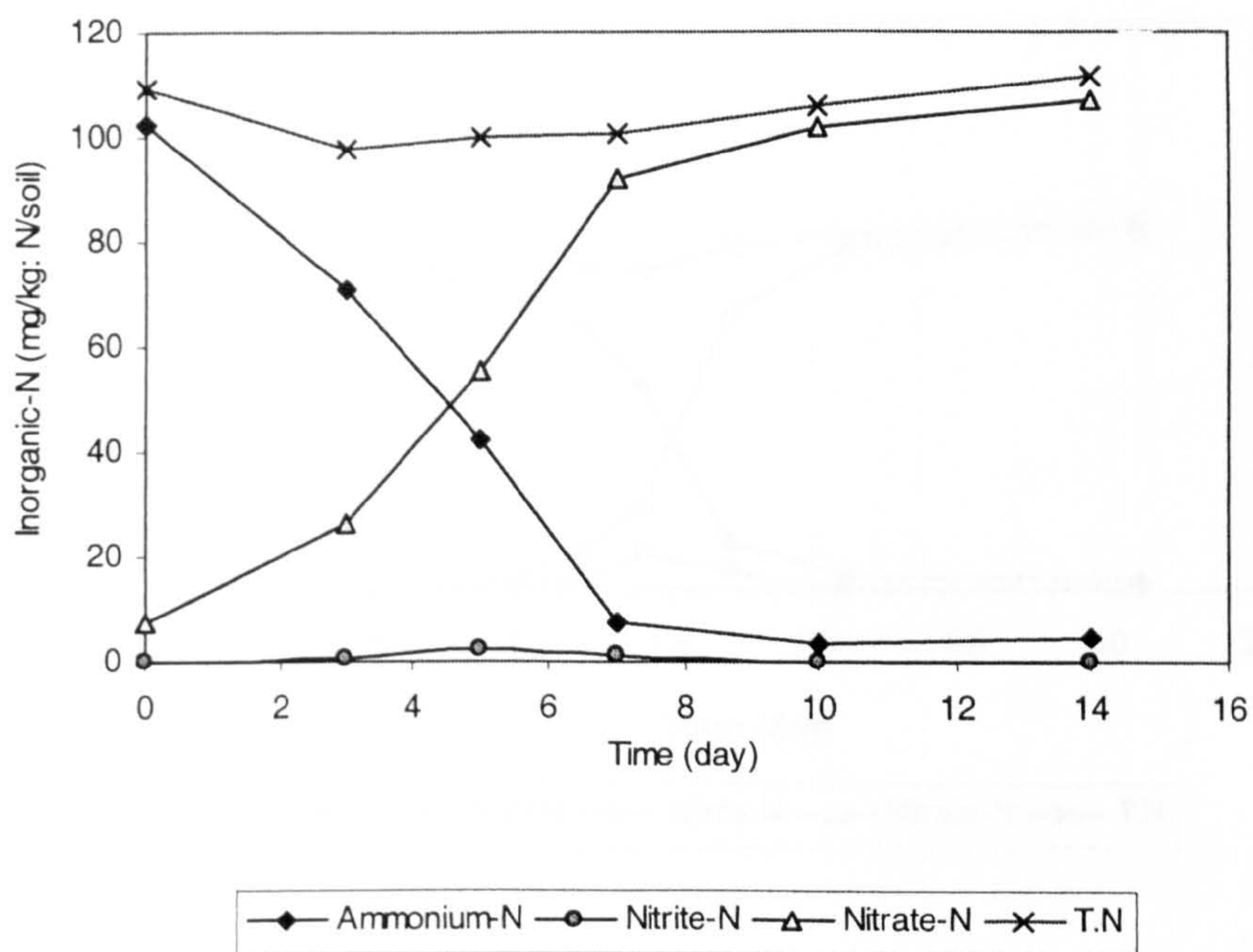


Figure 7.1: Changes in inorganic-N during nitrification incubation for soil 1 (mean of 4 replicates).

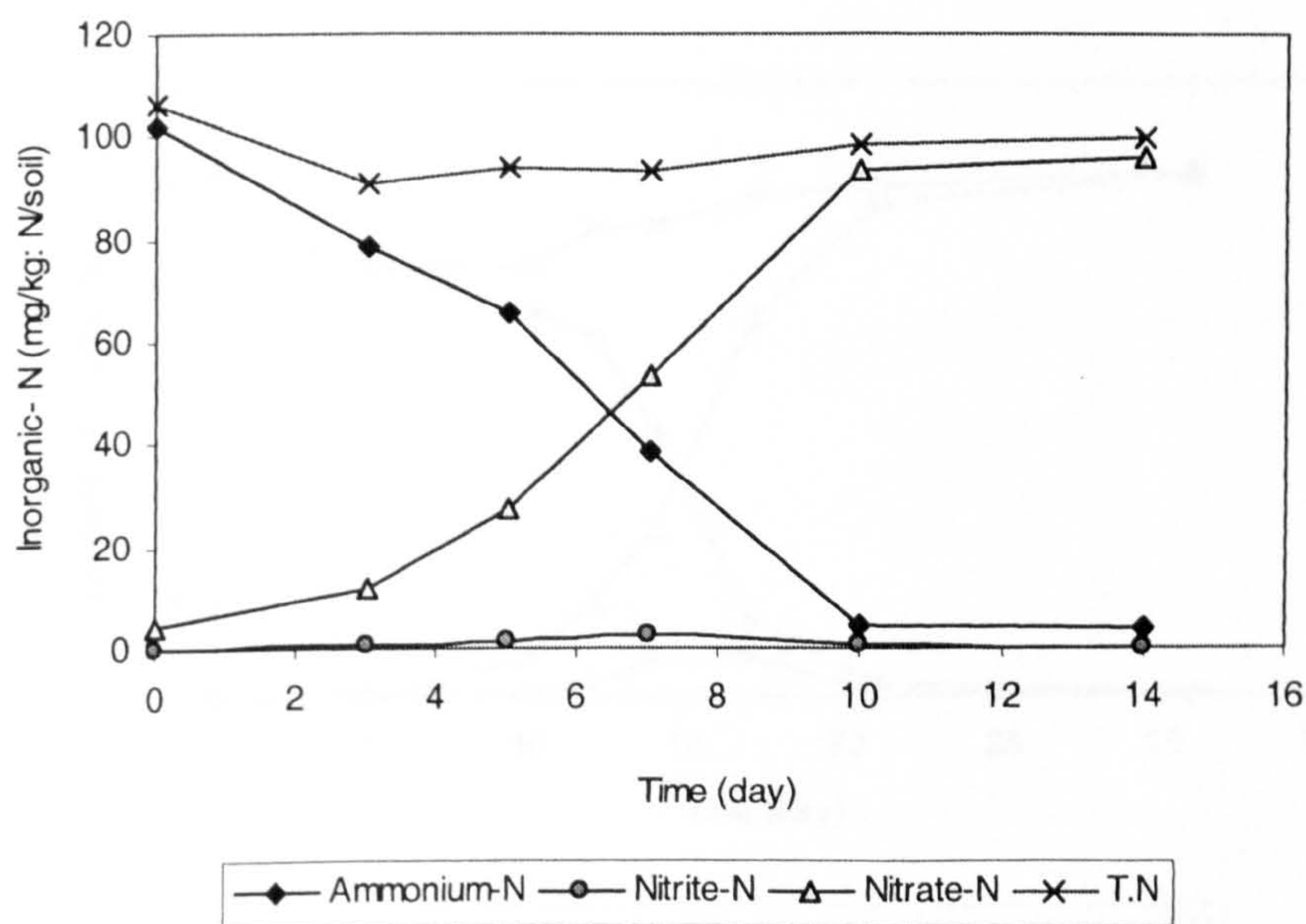


Figure 7.2: Changes in inorganic-N during nitrification incubation for soil 2 (mean of 4 replicates).



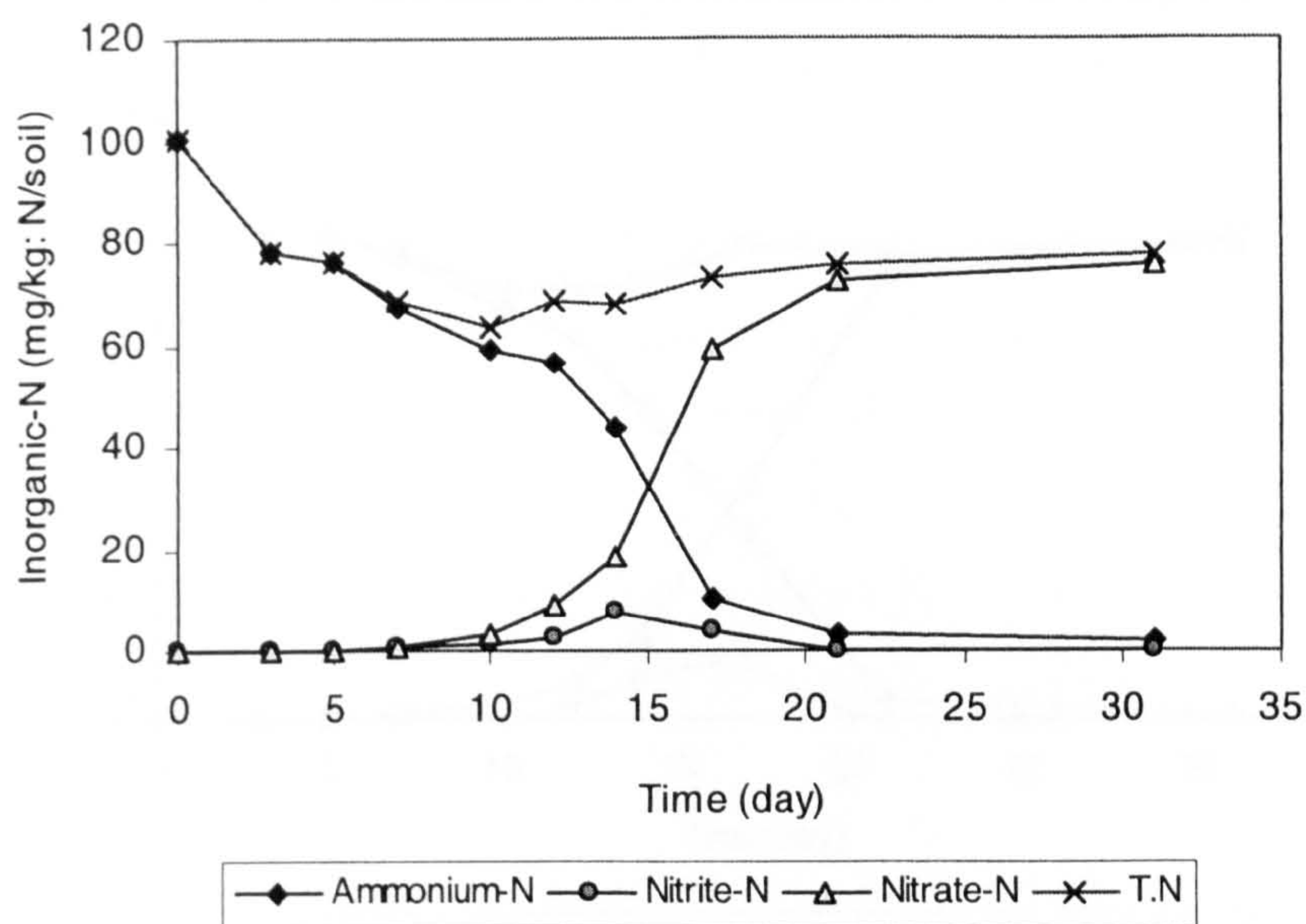


Figure 7.3: Changes in inorganic-N during nitrification incubation for soil 3 (mean of 4 replicates).

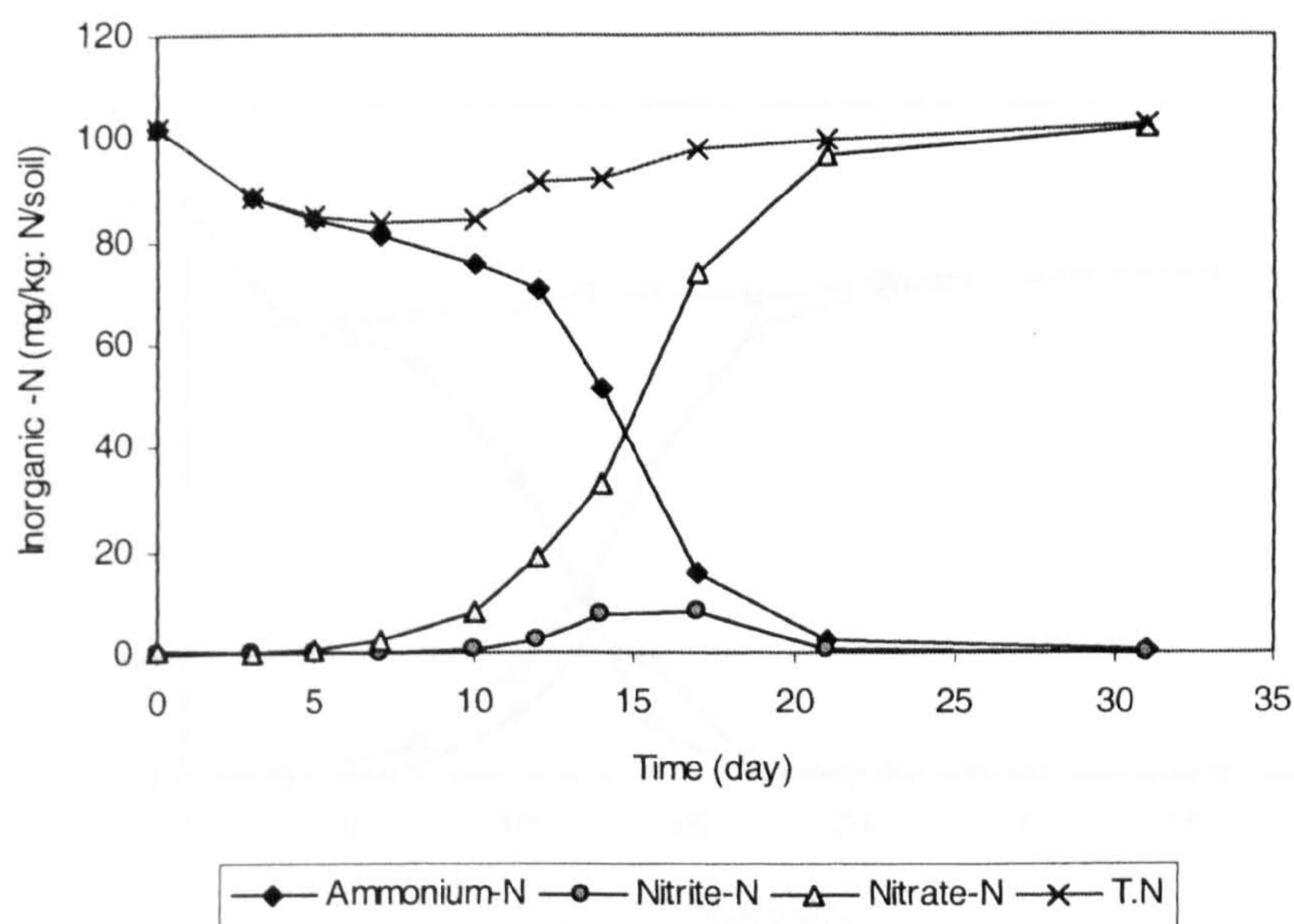


Figure 7.4: Changes in inorganic-N during nitrification incubation for soil 4 (mean of 4 replicates).

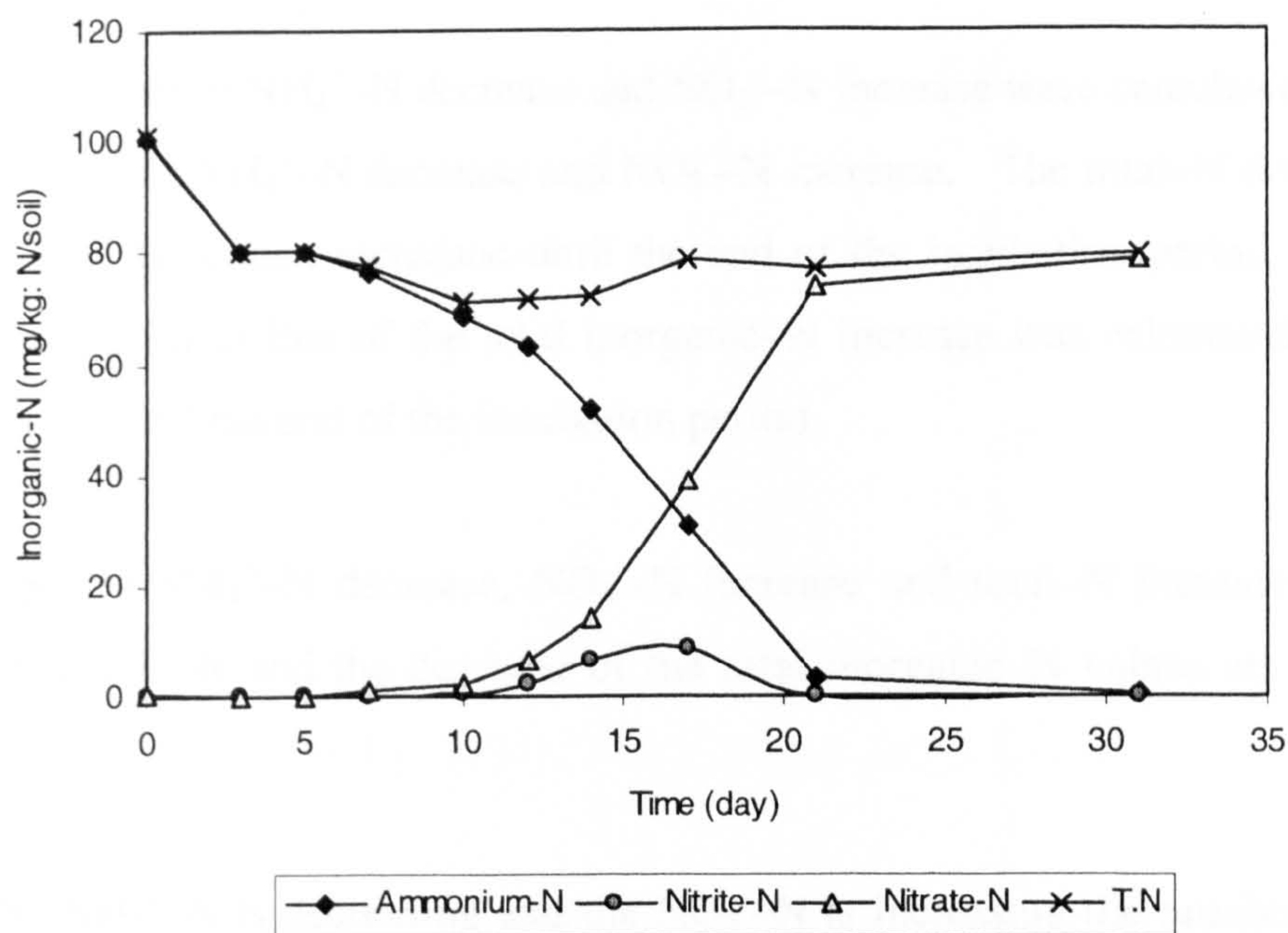


Figure 7.5: Changes in inorganic-N during nitrification incubation for soil 5 (mean of 4 replicates).

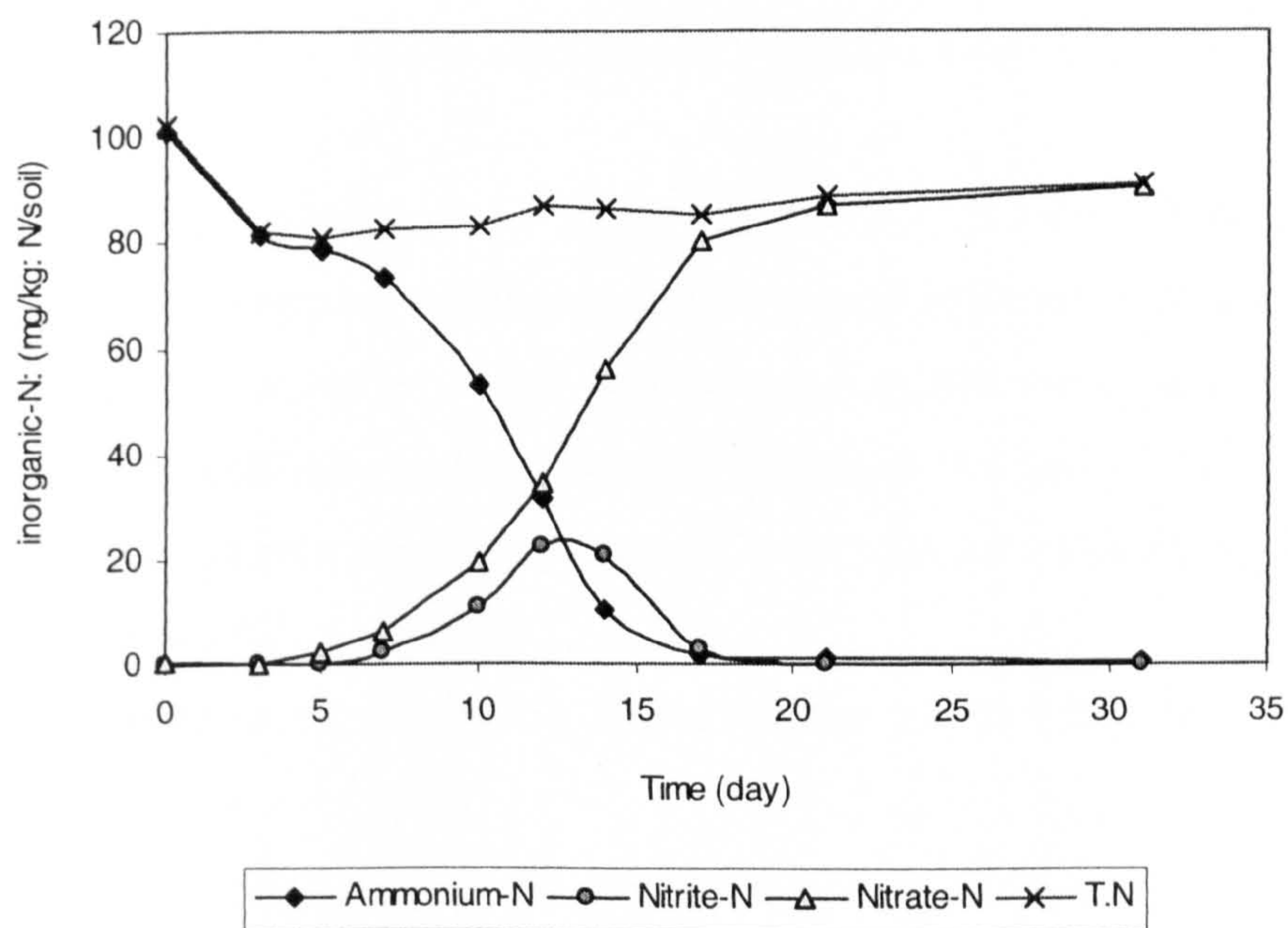


Figure 7.6: Changes in inorganic-N during nitrification incubation for soil 6 (mean of 4 replicates).



The rates (slope) of  $\text{NH}_4^+$ -N decrease and  $\text{NO}_3^-$ -N increase were calculated from the linear part of the  $\text{NH}_4^+$ -N decrease and  $\text{NO}_3^-$ -N increase. The total-N decreased in the first few days, then increased until the end of the incubation period. The rate (slope) of the linear line of the total inorganic-N increase was calculated from the lowest point until the end of the incubation period.

The slopes of  $\text{NH}_4^+$ -N decrease,  $\text{NO}_3^-$ -N increase and total-N increase and also initial inorganic-N and the decrease of the total inorganic-N values are shown in Table 7.2.

Since the  $\text{NH}_4^+$ -N is decreasing and the  $\text{NO}_3^-$ -N is increasing the number for their rates are negative and positive respectively. A comparison was made between the absolute values of the rate of  $\text{NH}_4^+$ -N decrease and  $\text{NO}_3^-$ -N increase for each soil using a paired t- test by Minitab.

The calculation of rates was from the linear part of the graphs, which are after the decrease phase of total inorganic-N. The rates of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N, were each compared within 6 soils using analysis of variance with Tukey HSD test.

Soil No	Slope ( mgN/ kg soil/ day)			Total-N
	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N		
1	-15.840 a Ψ	16.371 a Ψ	NS θ	1.253
2	-12.169 b	13.296 b	**	0.786
3	-11.115 c	13.607 b	**	0.620
4	-11.057 c	11.246 c	NS	0.844
5	-6.730 e	7.678 d	*	0.433
6	-7.650 d	7.745 d	NS	0.376

Table 7.2: The slopes (rates) of NH<sub>4</sub><sup>+</sup>-N decrease, NO<sub>3</sub><sup>-</sup>-N increase and change of the inorganic-N.

Ψ: Numbers with the same letters in column are not significantly different (Tukey HSD test), p> 0.05.

θ: Paired t-test between absolute values of rates of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N:

\*\* : Significantly different (p< 0.01).

\* : Significantly different (p< 0.05).

NS: Non Significantly different (p> 0.05).

Soil 1 shows the highest rate of nitrification and soils 5 and 6 have the least rates between the soils.

The rates of nitrogen production are slightly higher than the rates of the decrease in ammonium-N. This implies that there might be a small scale mineralization in these soils but statistical analysis of the data shows the differences are mostly non significant. The pH values were slightly reduced at the end of the incubation. Conversion of the ammonium-N to nitrate-N in the soils increases the acidity due to acid production during nitrification.

The soils extractable Cr is slightly higher in the extract from the end of the experiment.

Different researchers have obtained different rates for nitrification. They used different temperature and duration for incubation based on the soil variation. Some of these rates and conditions they obtained are summarized in Table 7.3



Author	Temperature °C and NH <sub>4</sub> application	Nitrification Rate mgN/kgsoil/day	Soil type
Flowers and O'Callagha 1983	15°C (50mg/kgsoil) (250mg/kg)	5- 6.57  2.27-5	Fresh field soil
Amin 1995	15°C (100mg/kg)	8.8-27.1	Fresh cultivated and garden soil
Metwally 1999	20°C (100mg/kg)	0.1-18	Six fresh soils
Watson 2000	13.5°C	3.25-13.26	Grassland
Badia 2000	25°C (1260mg/kg)	8.5 and 36.3	Gypsiferous and marl
Abbasi et al. 2001	20°C (200mg/kg fresh soil)	0-2.5cm 7.5 2.5-5cm 5.4 5-7.5cm 3.3	Fresh Grassland

Table 7.3: Nitrification rates obtained by different researchers.

As it can be observed grassland and arable soils show a wide range of nitrification rate. In the present study the nitrification rates were in the range of natural soils with soil 1 having the highest nitrification rate.

There was an accumulation of nitrite–N in all soils but, little in soils 1 and 2. Tate, 1995 mentioned that the most probable situation for accumulation of NO<sub>2</sub><sup>-</sup>-N concentration in alkaline condition is due to ammonia toxicity to *Nitrobacter*. Sauve et al. (1999) also mentioned that the nitrite oxidation step might be more sensitive to

metal toxicity than ammonia oxidation, which could potentially lead to accumulation of nitrite.

Hue and Adams (1984) conducted an incubation to study the minimum levels of inorganic phosphorus required for maximum activity of nitrifying bacteria in soils. They concluded from their results that the nitrification rates in soils were slowed by low P concentration in soil solutions. The effects were greater with  $\text{NH}_4^+\text{-N}$  than with  $\text{NO}_2^-\text{-N}$  oxidizing bacteria. They found a long lag period in ammonium oxidation and accumulation of  $\text{NO}_2^-\text{-N}$  due to delay of  $\text{NO}_2^-\text{-N}$  oxidation phase with low phosphorus concentration in soil solution. They reported that the minimum P level for the shortest lag period and the maximum  $\text{NH}_4^+$  oxidation was  $0.60 \pm 0.28 \mu\text{M}$ , and  $0.13 \mu\text{M}$  for the minimum delay time for  $\text{NO}_2^-\text{-N}$  oxidation and  $0.24 \mu\text{M}$  P for the maximum oxidation rate for  $\text{NO}_2^-\text{-N}$  oxidation. In the present study there was a significant correlation between available phosphorus and lag period in the soils of this area ( $r = -0.952$ ) which is illustrated in Figure 7.7 and Table 7.4.

The concentration of phosphorus in some parts of the site are low which are explained in chapter 4.

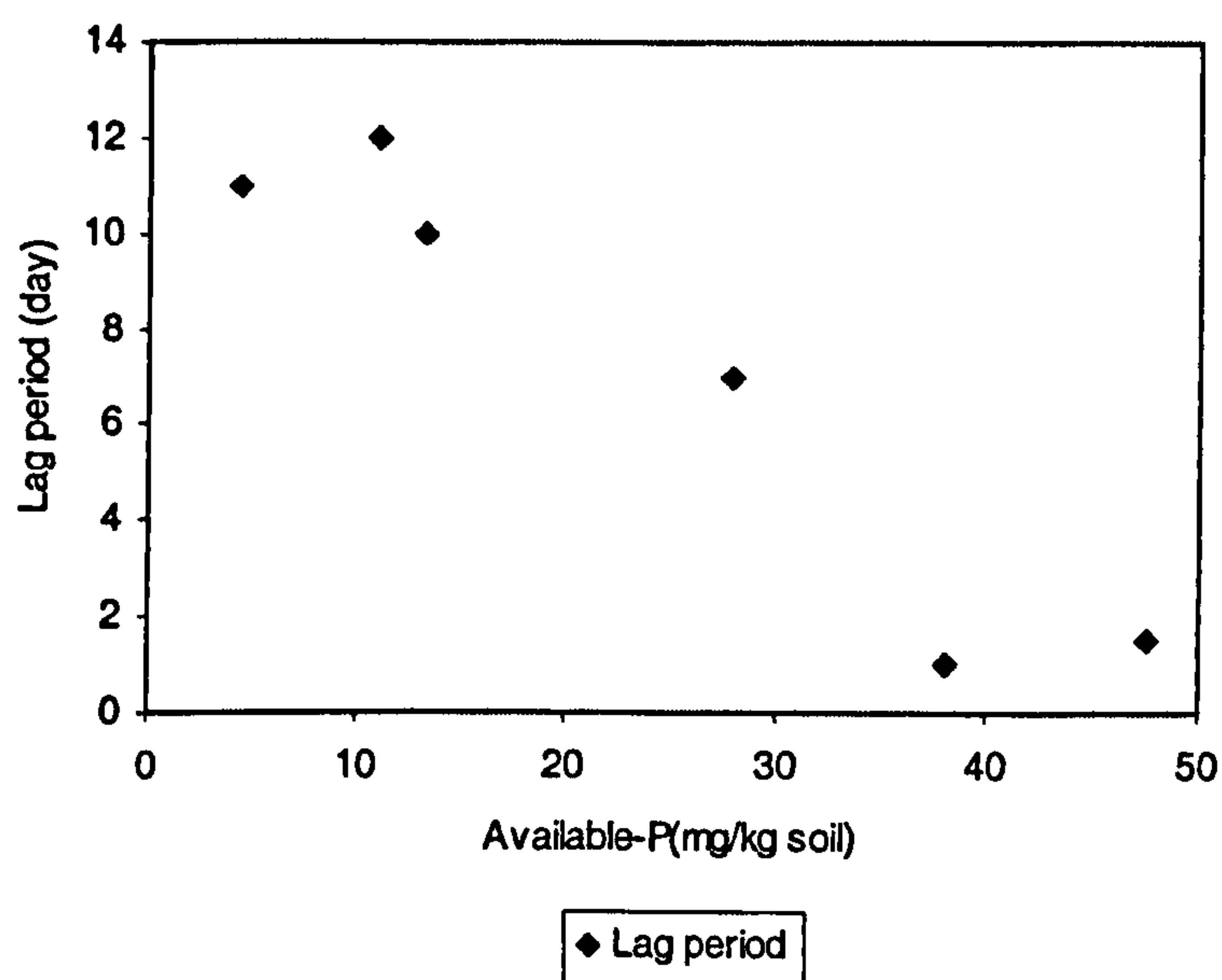


Figure 7.7: The relation between the lag period in the nitrification and the concentration of available  $\text{PO}_4\text{-P}$  in the soils.



Soil No	Available-P (mg/kg)	Lag period (day)
1	38.0	1
2	47.6	1.5
3	11.0	12
4	4.4	11
5	13.3	10
6	28.0	7

Table 7.4: Lag period and available–P in soils

Nitrification rates of the soils were sigmoid and can be divided to four phases. Firstly a lag period at the beginning of the incubation with no detectable nitrate production, secondly a gradual increase in production of the nitrate. Thirdly a linear maximum rate of nitrification. The fourth phase shows a falling rate. This subsequent slow rate of the nitrification, which is observed in all soils after the high rate, is probably due to  $\text{NH}_4^+\text{-N}$  limitation. These phases are illustrated in Figures 7.1-7.6. These stages of nitrification were also observed by Abbasi et al. (2001). The lag periods in present study were obtained by extrapolating back from the linear line of nitrate increase and were between 1-12 days (Table 7.4).

The delay of nitrification might be because the supply of ammonium-N from organic nitrogen mineralization maintains a small population of nitrifiers, which need time to increase to their maximum capacity upon the addition of inorganic  $\text{NH}_4^+\text{-N}$ . This suggest that there was a general increase in the population of nitrifiers with time, or that the nitrifying bacteria required a few days to built up their growth and to perform their function. The lag period was observed by Abbasi et al. (2001). In their study they investigated the mineralization and nitrification potential of a grassland ecosystem where they found a 7-14 days lag period for soils from different depth in the nitrification experiment.

They attributed this delay to immobilization and build up the population and growth of the bacteria. Sierra et al., (2001) also observed an 8 days lag period in their nitrification study of sludge amended soil and attributed it to the bacterial growth stage.

The maximum rate phase and lag period are considered to be good criteria for measuring environmental effect on nitrification. Flowers and O'Callaghan (1983) carried out an incubation study of soil with added ammonium sulphate. The environmental condition of their soils was not affected by limiting factors therefore they obtained a linear uninterrupted graph without delay or lag period coming directly from the beginning of the incubation.

The first phase of the total inorganic-N in all soils declines and shows that the inorganic nitrogen losses happening in the soils in the first few days.

In the mineralization incubation there was no detectable ammonia gas trapped in the acid solution during the incubation period because there was no detectable ammonium-N in the soil extracts. Since in some soils  $\text{NO}_3^-$ -N was produced during mineralization incubation period, it suggests that any ammonium-N produced in the mineralization incubation was converted to nitrate-N immediately and was not available for gas volatilization. In the nitrification incubation ammonium-N was added to the soils. Therefore it could be a possibility of occurrence of ammonia gas volatilization during this incubation due to the presence of high concentrations of ammonium-N.



### 7.3.2 Second Incubation

The N-balance in the soils in the second incubation: initial total inorganic-N, total inorganic-N after 10 days, total inorganic-N decrease, NH<sub>3</sub> trapped by hydrochloric acid during incubation and possible immobilization are shown in Table 7.5.

Soil No	mg/kg: N/soil				
	Total -N at time zero	Total -N after 10 days	Total - N decrease	Volatilized in acid (mg NH <sub>4</sub> <sup>+</sup> -N /kg soil)	Immobilization
1	122	90	32	14	18
2	109	81	28	16	12
3	101	67	34	13	21
4	101	77	24	3	21
5	100	70	30	14	16
6	101	74	27	18	9

Table 7.5: The inorganic nitrogen budget in soils in the short-term nitrification incubation.

It is clearly observed that the total inorganic-N has decreased considerably in all soils.

The values of NH<sub>4</sub><sup>+</sup>-N in the acid solutions indicates that the soils lost NH<sub>4</sub><sup>+</sup>-N as ammonia gas to different extents. This is presumably caused due to high pH of these soils. Roelcke et al. (1996) conducted a study of ammonia volatilization of soils. They observed that the considerable amount of ammonia is volatilized from the soils containing about 10% CaCO<sub>3</sub> and pH 7.7. They attributed the nitrogen volatilization to high pH of the soil. They also mentioned that in addition to high pH, a low bulk density increases the amount of ammonia-N diffusing towards the soil surface. Ammonia volatilization is lower in more compact soils with higher density.

From Table 7.5 it can be observed that the extent of inorganic nitrogen loss is greater than can be accounted for an ammonia gas loss.

Denitrification is a process in which nitrate or nitrite produced in soil is reduced to gaseous nitrogen like  $N_2$  or  $N_2O$  and  $NO$ . This phenomenon occurs when anaerobic conditions occur due to high water content of soil. In this experiment the moisture content of soil was in the level of  $-0.5$  bar which maintains an aerobic condition during the period of the experiment. The  $-0.5$  bar moisture content of incubated soil, is an optimum moisture level for microbial activity where microorganism growth and metabolism is not limited by water, and there will be sufficient  $O_2$  for aerobic condition. Therefore denitrification should not have occurred in these soils. For that reason further to ammonia gas volatilization, immobilization might be another cause of nitrogen loss in the beginning of the incubation. During the incubation conducted for a nitrogen mineralization study of these soils a total-N decrease also occurred in the first week of the experiment which was due to immobilization. This was followed by the 2-3 weeks lag period in some soils with no nitrogen mineralization in others that was attributed with high C:N values of the soils.

Sauve et al. (1999) studied the effects of Pb and Cu on nitrification of contaminated soils. They mentioned that the pH and soil organic matter are the most influential parameters in these soils. They pointed out that the total metal content of soil is not sufficient to explain the variation in nitrification rates. This could be due to a combination of direct and indirect effects. Nitrification could be influenced by the total metal concentration but the bioavailability of total soil metals are affected by soil properties determining speciation, thus soil properties may affect nitrification directly or indirectly through effects on metal speciation.

In the present study the total concentration of chromium in soils is very high and total nickel is also higher than threshold values, but the high pH provides the situation that very small amounts of chromium and nickel are available in these soils.

Binding of metal to organic material, precipitation, complexation, and ionic interactions are important to availability. pH can have considerable effect on the availability and thus the toxicity of heavy metals in a given environment. The total chromium content of the soil of this site is generally very high. The majority of samples contain more than  $1000\text{mgCr/kg}$  soil. There were some samples, which contained more than  $10,000\text{mgCr/kg}$  soil. However, water extractable chromium in



these soils, especially the surface soils, was not high. Soil water extractable Cr is Cr(VI). This implies that the total chromium in these soils is in the form of Cr(III). Cr(III) can complex with some organic ligands such as fulvic acid, citric acid, diethylene triaminepentaacetate (DTPA). These complexes are soluble above pH 5.5, while non complex Cr(III) is precipitated above this pH (James and Bartlett, 1983a). Liming the soil decreases the extractable Cr(III) in soil. That is because liming forms inorganic hydroxides from Cr(III) and prevents the organic complexes formation (James and Bartlett, 1988).

The soils of the research site have high pH and high content of carbonate. Therefore presumably the chromium content in these soils is in the precipitated form of hydroxide or carbonate which is not bioavailable.

## 7.4 Conclusion

From the experiments above it can be concluded that all soils show the existence of nitrification of added ammonium-N after a lag period. Despite the existence of high total chromium, the available chromium in this soils is low. Sauve et al. (1999) also pointed out that the total metal content of soil is not a sufficient guide to show limitation of the nitrification process. After a period of total-N decrease, a lag period occurred in the incubation with no detectable nitrate-N production, which was attributed to immobilization and build up of the population and growth of the bacteria. The length of the lag period was negatively correlated to soil extractable phosphorus ( $r = -0.952$ ). A lag period also was observed by Abbasi et al. (2001) and Sierra et al. (2001), and attributed to build up the population and growth of bacteria. Hue and Adams (1984) also observed the correlation between soil available phosphorus and lag period in nitrification experiment.

Loss of inorganic-N was observed during the first days of incubation, which was partly due to ammonia gas volatilization. The immobilization of inorganic nitrogen is also believed to have happened during the first few days presumably because of a large amount of soluble C present with high C:N in the soils.

Overall the situation in the soils shows a nitrogen poor system in the soils but nitrification in these soils indicates that these soils have a potential for the nitrification process provided  $\text{NH}_4^+\text{-N}$  is available.



# CHAPTER EIGHT

## GENERAL DISCUSSION AND CONCLUSIONS

### 8.1 Introduction

There is an increasing concern about the natural environment and the need to protect it for the benefit of future generations. Derelict land forms a great part of the environment as old industries ceased operation. The potential hazards, which can arise from the addition of chemical substances to soils, are probably not a matter of dispute. New businesses prefer to establish their operation on a green field and reclaimed soil rather than contaminated site. Therefore it is necessary a remedial action to the contaminated sites to overcome the problem before any further developments could take place, and to render the sites safe.

Removal or treatment to negate the effect of contamination can be a stage of the reclamation process. In many cases it becomes necessary to establish a new and viable use of the land. Sites may become available for housing, commercial or industrial redevelopment. They also may be required to support agriculture, forestry or recreational uses (Cairney and Hobson, 1998).

Some parameters should be considered for remediation action:

Variation: Site specific variation is inevitable on a contaminated site, particularly when the land might have been used for a range of industrial processes over a century or more. These variations can impose limits on the use of some techniques, but are of less importance to the other methods.

Cost effectiveness: Since contamination could limit the land's use and value, remediation is undertaken to remove this problem. However the final end value of the reclaimed site usually imposes economic limits, which in turn encourage the use of simpler and cheaper remediation methods.

Speed of reclamation: The sooner the reclamation is completed, the quicker can the redevelopment proceed and a return on the initial investment is obtained (Cairney and Hobson, 1998).

Metals in particular occur as a wide range of possible species and to a greater or lesser extent. Thus solubilities and bioavailabilities differ and a concentration of a metal as one particular species might have no significant risk, while the same concentration of another species might pose substantial risk to people using the land. Factors such pH, redox potential and the amount of clay minerals and organic matter affect whether or not contaminants are locked away or are easily available for uptake to create risks (Alloway, 1995).

Chromium has been known as a pollutant of the environment despite its role in human metabolism. The chromium released to the environment can be from anthropogenic source or natural source. Human activities include sewage sludge application to agriculture, metallurgical industries and refractory brick production. Windblown soil and volcanic are the major natural resources of chromium in the environment (Nieboer et al., 1988).

Metallic contaminants tend to be most concentrated in finer particles of soils. If the contaminated site is used for walking or games, then the bare soil could cause high chromium dust problems to occur in dry weather, which is toxic and in some cases carcinogenic. The contamination of groundwater by chromium can also be important in industrial areas. Chromium can also enter surface water and is toxic to fish. It is able to damage the growth of plants and microorganisms. Cheaper solutions to overcome the problem can be aimed at breaking the link between source of the contamination and the target. Vegetation cover can reduce the runoff water and contaminant to be carried out by surface water. It also minimizes the dust in dry weather and decreases the risk by inhalation. It can also decrease the leaching of contaminant to ground water. Trees and bushes can discourage access to the area.

There is not ever two identical sites. Each will require to be appraised on its own merits and specific methodology determined. The basic requirements of all plants



are sunlight for photosynthesis, water, oxygen and nutrients. The presence of topsoil is important and is needed for planting.

## **8.2 Study of a Chromium Contaminated Site**

A site, situated at the end of Summerford Road, just off Windsor Road in Falkirk is the study area for this project (Figure 4.1). This site has had three main uses over the last 150 years. It was occupied by a chemical works at the western end of the site. The works mainly dealt with the production of chromium salts. They were produced from the chromium iron ore, chromite. In this process ore was finely ground and mixed with calcium carbonate. The processes, which then led to production of potassium chromate, left precipitated calcium carbonate after the work. Finally the process produced a calcium carbonate waste which contained 1 to 3%  $\text{CrO}_3$ , and is equivalent of 5,000-15,000mg/kg Cr in  $\text{CaCO}_3$ .

The slag tip (heap) remained in the area. An iron works and a concrete processing plants were another two former main uses of this site. In 1977 Central Regional Council took over the site and called in contractors to demolish the existing buildings. They then bulldozed them up against the side of the tip. The top of the tip was then leveled off by pushing the excess material over the top of the concrete waste rubble, which formed a fairly steep slope down to the flat area of the site. Therefore the area has three different parts. One part is located at the flat top of the tip, the second part is a slope area and the third is another flat part at the bottom of the slope. The council sold two corners of the site for an old people's home and for private housing (J. Stewart, personal communication).

The poor vegetation and poor establishment of trees in the central part of the site on the spoil heap area led to this investigation of the area. The primary investigation showed pools of water with a yellow crystalline deposit with pH 9 and 60mg/l of soluble chromium going to the road and the storm drain carrying some sediment from the area. Water with a high level of chromium and a yellow colour can also be leached from the site especially from the different layers of the slope and can increase the risk of the exposure of the chromium to the children and adults. In order to reduce this risk a vegetation cover is needed for this site.

The understanding of the total nutrient conditions in plant and soil of this former industrial contaminated land is important for better reconstruction and vegetation establishment of the site. A soil survey, a pot experiment and different incubation studies have been carried out to understand the nutrient and growth potential and probable chromium toxicity on this site.

From the survey study of the site, soils were shown to be contaminated with chromium and have a low nitrogen content. The pot experiment proved that nitrogen is the limiting factor for plant growth and that when sufficient is applied, then P or compaction might be the next limiting factor. In the study of nitrogen turnover in soil from this chromium contaminated site, nitrogen mineralization was shown to be low compared with natural grassland soils, while nitrification rate is comparable with natural soil provided  $\text{NH}_4^+$  is available.

In the present study effort has been focussed on nitrogen transformations. During the mineralization incubation study, no net phosphorus mineralization occurred in these soils, which implies that any phosphorus mineralized is used by microorganisms in the soils. Moreover in the nitrification incubation there was a lag period after the initial nitrogen loss with no detectable nitrate produced. This was attributed to the built up of the bacteria population. There was a significant negative correlation between available phosphorus and the length of the lag period in the soils (-0.952), therefore phosphorus could be another limitation for the bacteria. This shows a need for further investigation on these soils from the phosphorus point of view.

In the pot experiment, soil from the area with the poorest level of available phosphorus showed less response to nitrogen fertiliser. This might be due to low phosphorus content of the soils despite the application 50 kg/ha P along with different levels of nitrogen. This might lead to earlier decline of the nitrogen response curve for this soil.

From the pot experiments it was observed that the application of 100 /50 /50 of N/P/K fertilizer could obtain a maximum yield for the site.

During plant residue decomposition assimilation of C and N occur simultaneously. The C assimilation rate depends on the rate of decomposition of plant material and



the assimilation yield of the decomposed C by the microflora. The N assimilation then determined by this carbon flow and C:N ratio of decomposer. The decomposition of compounds with high C:N ratio results in nitrogen deficiency. This is because microbes (bacteria) have C:N ratio 4/1 to 6/1 and fungi have ratio from 10/1 to 12/1 and some mineralized nitrogen must be used by microbial community to balance this need for new microbial biomass (Tate, 1995; Wong et al., 1998)

Any treatment for this site as a contaminated site must be long lasting. The nitrogen turnover being low shows the necessity to establish a nitrogen cycle capable of maintaining continuous vegetation cover for the site. Adding chemical fertilizers may lead to higher cost and a short term recovery of vegetation. Fertilizer nitrogen may also volatilize quickly from the site due to the high pH. Therefore it is not recommended to add chemical fertilizer year after year due to high cost and short term efficiency. N cycle should be well established to support the vegetation growth and microbial activity. Zaman et al. (1999) stated that the application of inorganic source of ammonium  $\text{NH}_4^+$  did not increase nitrogen mineralization while dairy shed effluent (DSE) promoted mineralization because of the presence of readily mineralizable organic substances with low C:N ratio and stimulation of soil microbial activity. The application of organic source of nitrogen can help to promote nitrogen mineralization and eventually better nitrogen turnover.

Singa et al. (1998) mentioned that sewage sludge is a suitable organic manure because it serves as an efficient source of nitrogen due to its high N content and faster mineralization rate.

Wong et al. (1998) pointed out that sewage sludge contains a substantial amount of nutrient such as C, N, P and trace elements, which are essential nutrients for plant growth. Due to the large amount of organic matter in sludge it can act as a soil conditioner to improve the physical properties of soil such as soil aeration and water holding capacity. This is particularly useful for soils with poor physical condition like compact soil or sandy soil.

To reduce the loss of nitrogen derived from nitrification on the ground by leaching it is better to maintain the nitrate availability at the level which is required for plant

growth. This can be achieved by using a slow-release nitrogen source. Ammonium concentration is a primary limitation of nitrification. Once it forms it rapidly nitrifies to nitrate. Therefore sewage sludge may be used to alleviate the nutrient condition for better growth. Sewage sludge was applied on one part of the site earlier. It helped and resulted in better vegetation. Applying to the whole site can promote better nitrogen mineralization and subsequent nitrification of ammonium released over the contaminated site for improving the vegetation.

Nitrogen fixation is another essential input to the soil nitrogen cycle. Use of plants capable of nitrogen fixation, such as legumes, can improve development of reclamation and management in damaged or mismanaged soils because the fixed organic nitrogen is mineralized later and helps to improve the nitrogen cycle in this kind of soil.

In the present study the soils which were used for the fertilizer response curves and incubation experiments were all surface soils (0-15cm). In the pot experiments these soils were shown to grow good root systems, which are able to absorb nutrients, but on the site there is a relatively hard waste material containing chromium in subsurface soil, which might damage the root system. Before sampling 2-3cm of surface soil and root mat were removed. This could help to the nutrient cycling, availability and growth under field condition. Under most circumstances, particularly in grassland the surface of the soil profile represents the zone of maximum biological transformations (root, microbiological and soil faunal activity) where interaction between environmental changes and nitrogen inputs and removal is also likely to be at maximum.

### **8.3 Analytical Approaches**

Since the project included plant analysis building on the study of a former student (Amin, 1995) in this department, work has been done on phosphorus analysis. Moreover during the study of the contaminated site some analytical problems were faced.



Chromium was shown to interfere with colorimetric analysis of nitrite-N and nitrate-N. The systems are more sensitive to first addition of chromium, and caused overestimation of nitrate-N and nitrite-N. This overestimation can cause big errors in solutions with low concentrations of nitrite-N and nitrate-N. A new system was developed with addition the chromium to the reagent which could reduce the interferences. Using this new system, allowed chromium contaminated soil extract solutions to be run for nitrite-N and nitrate-N with negligible overestimation. This system is particularly useful when low amount of nitrate-N and nitrite-N are analysed.

In a broad comparison of different digestion methods and analysis of phosphorus in plant -P, perchloric acid digestion method and ascorbic acid analysis method are shown to give the better and reliable result .

## 8.4 Other Reclamation Options

Hyperaccumulators: Plant growing in non contaminated soils contain non detectable or lower amount of metals compared with those growing on soils with higher concentration of metals. There are some plants that are able to grow on contaminated soils and absorb higher amount of metals, which are called hyperaccumulators. There has been interest in cropping and removing the above ground biomass of hyperaccumulators of metals for decontamination of polluted soils. The metal content of plants is related to concentration of available form of metal in the soil. The availability of metal in the soils is affected by soil pH, redox potential, clay and organic matter (Alloway, 1995). Phytoremediation is defined as the use of green plants to remove pollutant from the environment or to render them harmless and is a potential cost effective remediation for many contaminated sites. Phytoremediation cannot be used for remediation of chromium contaminated sites because chromium absorbed to the plants is not mobile and accumulated in root system.

Reduction: Reduction of hexavalent chromium to the trivalent form would lower chromium bioavailability and toxicity to environmental and biological system.

Chromate detoxification can be carried out chemically with reducing agents such as  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$ . However chemical reduction methods are often not cost effective and may themselves produce hazardous by-product. Microorganisms are able of altering the redox state of toxic metals through direct and indirect biological and chemical processes (Smith and Gadd, 2000).

The processes of biological oxidation and reduction of sulfur compounds in the biosphere are closely related to the mobilization and immobilization of metals in biochemical cycles (Withe et al., 1998). Sulfur reducing bacteria are obligate anaerobic heterotrophs which couple oxidation of a carbon/energy source to sulphate reduction. The sulphate is reduced to sulphide, which can interact with many toxic metal to precipitate insoluble metal sulphide and this serves as a basis for several bioremediation treatment methods. Cr(VI) reduction and precipitation by sulphate-reducing bacteria was observed (Salunkhe et al., 1998), (Smith and Gadd, 2000).

There are also other techniques for remediation of contaminated land but there might be more expensive than establishment of vegetation cover. However the final end value of the reclaimed site usually imposes economic decision.

Clean cover: This technique consist of a thick clean materials which is laid over a contaminated site to separate the proposed re-use material from whatever contaminated still exist at depth. A cover mainly intended to combat the upward movement of contaminated soil moisture. The effectiveness of this cover may decline with time.

Dilution of contaminant: This technique is useful where contamination is uneven, where only a thin capping of contaminant material overlies clean natural soil. The contaminant can be mixed with natural soil to dilute the concentration of contaminant under the threshold value such as ICRCL.

On site encapsulation: In this method the surface contamination scraped off and deposited in a capped mound. For this purpose the contaminated waste is compacted to ensure that the surface area available for leaching will be minimal.



Excavation and disposal: This way is an off-site disposal to a licensed tip for use as reclamation solution. This type of action may exhibit a very wide range of possible hazardous materials such as metal rich dust, toxic gases and vapour to workmen and people living in that area.

Soil dry screening: when a site's soils necessarily have to be excavated to remove troublesome layers and deposits, the opportunity exists to amend and improve the chemical nature of the excavated materials and possibly re-use most of the dug soils. The basis on which dry screening relies is that metallic contaminant (in particular) tend to be most concentrated in the finer particles of soils. Thus a prior sieving trial, on a large and typical soil sample can, in most cases, identify which particle size distinguishes "clean" from "contaminated" soil.

Soils washing and stabilization are also techniques, which are likely to be appropriate for metal contaminated sites (Cairney and Hobson, 1998).

## **8.5 Further research consideration**

- In the present study effort has been made on nitrogen transformation in these soils. Since the available phosphorus and lag period in the nitrification of this site was highly correlated it shows the need for further investigation on these soils from the phosphorus point of view.
- It is assumed that the plant availability of different metals existing in the soils is not high due to metal speciation at higher pH. In order to confirm this the further investigation of Ni and other heavy metals in these soils is also recommended. The soil survey showed total-Ni content of these soils mostly higher than ICRCL guideline. Cr and Ni often appear together in rocks such as serpentine and some minerals ores. In some cases Ni tend to be more contaminant than Cr. Ni is also a mobile metal in plant.
- Chemical fertilizers were used in this study for incubation experiment. It is suggested that the incubation study to be carried out using different organic manures such as sewage sludge or chicken manure to look at mineralization, nitrification and volatilization.

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