Nitrogen dynamics in sewage sludge and commercial fertilizer enriched soils

by

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DECLARATION

I declare that this thesis, which I am submitting to the University of Pretoria for the degree, M.Sc. Soil Chemistry, is entirely my own original research, except where acknowledged, and has never been submitted to any other tertiary institution for any degree.

Signed: C.v. Niekerk Date: Februarie 2004

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ABSTRACT

This study indicated that sewage sludge could be used as an alternative to commercial inorganic fertilizers.

It is common practice in South Africa to use agricultural land for disposal of sewage sludge. The disposal of sludge must however be done in a responsible manner to avoid environmental pollution such as nitrate (NO_3^-) leaching. In South Africa strict guidelines exist regarding sludge disposal, and a maximum of 8 ton ha^{-1} $year^{-1}$ (dry mass basis) sludge may be applied. This value was based on possible NO_3^- leaching, but no equivalent legislation exists for commercial fertilizer that could result in the same harmful effects. In this study the possible pollution hazard in terms of NO_3^- leaching from sewage sludge was investigated and compared to commercial fertilizer.

An incubation trial was done to determine the mineralisation rate of sludge and fertilizer. The rate at which inorganic N, NO_3^- and NH_4^+ was produced from sewage sludge was measured during this experiment. Sludge was applied at three different loads: 5, 10 and 20 ton_{dry} ha^{-1}. Commercial fertilizer was also applied on three different levels and each level was equivalent to 30% of the N content of the corresponding sludge treatments. It was found that in the sludge treatments the NH_4^+ levels immediately increased possibly due to microbial activity. The NH_4^+ levels reached a maximum on day 7, with a production rate of 14, 26 and 60 mg kg^{-1} NH_4^+ for the 5, 10 and 20 ton_{dry} ha^{-1} treatment, respectively. After day 7, the production rate decreased while the NO_3^- production started increasing at the same rate at which the NH_4^+ levels decreased, as a result of nitrification. The 5, 10 and 20 ton_{dry} ha^{-1} did not show any increases in NO_3^- production initially, but production started increasing on day 7 and stabilised after 28 days.

In the fertilizer treatments the NH_4^+ levels increased immediately after application. The levels decreased again due to nitrification. Unlike the sludge treatments, an immediate increase in NO_3^- production was observed. This is because of the

inorganic nature of the applied fertilizer. The NO_3^- content increased over time, and at the same time the NH_4^+ content decreased. All the inorganic N from the fertilizer is immediately available, while the N in sludge must first be mineralised before the inorganic fraction becomes available. NO_3^- production from sludge is steady, and after day 28 the total NO_3^- production from sludge exceeds the total production from fertilizer. Even though more NO_3^- (mg kg^{-1}) was produced from sludge, the distribution was different and could be utilised more effectively by plants. The risk of NO_3^- leaching from commercial fertilizer is therefore possibly more than the risk of NO_3^- leaching from sewage sludge applications.

To correlate the above laboratory mineralisation values to that of field conditions, a field trial was done. The potential leaching and the effect of plant uptake were also investigated. Two trials were done: one for winter and one for summer conditions. Each trial continued for three months, and soil samples were collected every two weeks on depths of 0-30 cm, 30-60 cm and 60-90 cm. For the winter trial, application levels of 4, 8 and 16 ton_{dry} ha^{-1} sludge were applied. Low mineralisation rates and subsequently low NO_3^- and NH_4^+ levels were measured that could be attributed to low microbial activity. No NO_3^- leaching was detected. To obtain better results the application rates were adjusted to 20 ton_{dry} ha^{-1} sludge and corresponding fertilizer treatments during the summer trial. Maize was used as a crop to measure the effect of plant uptake on NO_3^- leaching. No significant differences were obtained between the open blocks and the maize blocks. Significant leaching occurred under fertilizer treatments, but none under sludge treatments. Mineralisation was also much slower in the field trial than in the laboratory trial, and after 90 days, there was still NH_4^+ production in the top horizons.

A part of the organic N in sludge was lost through denitrification. These losses could reduce the available inorganic N and subsequently reduce the risk of NO_3^- leaching. An incubation study was done to measure the gaseous losses of N through N_2O and N_2. Gas samples were taken and measured on a gas chromatograph. Similar application rates that were used in the previous incubation study were applied, as well as different moisture contents. N_2O production from sludge increased immediately after sludge application, after which the production rate steadily decreased until day

14. No significant differences were obtain between 50% and 100% moisture content, and very little N_2O production was found from the fertilizer treatments.

N kinetics were done on the data obtained from the incubation studies. These values indicate the rate at which sludge could be mineralised and the subsequent rate at which inorganic N, such as NO_3^- were produced. N balances were also drawn to indicate the quantity of organic N that is mineralised, as well as the change between different inorganic N fractions over time.

This study compared the potential N pollution from sewage sludge and commercial fertilizer in agricultural soils. By using all the results mentioned above, a better idea on the dynamics of sewages sludge compared to commercial fertilizer could be obtained. This knowledge could assist to apply sludge as a fertilizer to achieve the maximum benefit from the N content in the sewage sludge, without detrimental environment impact such ass groundwater contamination.

OPSOMMING

Hierdie studie dui aan dat rioolslyk 'n meer geskikte alternatiewe kunsmis bron mag wees as kommersiële kunsmisstowwe.

In Suid-Afrika is dit 'n algemene praktyk om rioolslyk as kunsmis te benut. Die toediening van slyk moet egter op 'n verantwoordelike wyse geskied, om sodoende omgewingsbesoedeling, soos nitraat (NO_3^-) loging te voorkom. Die slykriglyne in Suid-Afrika dui 'n maksimum slyk toediening aan van 8 ton ha^{-1} $jaar^{-1}$ (droë massa basis). Hierdie waarde is gebaseer op moontlike NO_3^- loging, maar geen ekwivalente riglyne bestaan vir die toediening van kommersiële kunsmisstowwe, wat dieselfde negatiewe oorsake tot gevolg kan hê. In hierdie studie word die moontlike besoedeling in terme van NO_3^- loging vanaf rioolslyk bestudeer, en vergelyk met kommersiële kunsmis.

'n Inkubasieproef is gedoen om die tempo waarteen anorganiese N (NO_3^- en NH_4^+) vanaf riool slyk geproduseer word, te bepaal. Slyk is toegedien in drie verskillende ladings: 5, 10 en 20 $ton_{droë}$ ha^{-1}. Kommersiële kunsmis is ook in drie ladingsvlakke toegedien, elke vlak ekwivalent aan 30% van die totale N inhoud van die ooreenkomstige slyk behandelings.

In die slyk behandeling verhoog die NH_4^+ vlakke, waarskynlik as gevolg van mikrobiese aktiwiteit, terwyl dit by die kunsmis behandeling verhoog as gevolg van die anorganiese aard van die toegediende kunsmis. Die NH_4^+ vlakke in die slyk bereik 'n maksimum op dag 7 van 14, 26 en 60 mg kg^{-1} NH_4^+ in die 5, 10 en 20 ton ha^{-1} behandelings onderskeidelik. Na dag 7 verlaag die produksie-tempo van NH_4^+ terwyl die produksie-tempo van NO_3^- verhoog teen dieselfde tempo waarteen NH_4^+ afneem, as gevolg van nitrifikasie. Die 5, 10 en 20 ton ha^{-1} slyk behandelings toon aanvanklik geen toename in NO_3^- produksie nie, maar na dag 7 begin produksie toeneem, en stabiliseer na dag 28.

In die kunsmis behandeling is daar 'n onmiddelike verhoging in NH_4^+ vlakke, en die NH_4^+ vlakke neem weer dadelik af as gevolg van nitrifikasie. Anders as by die slyk behandeling, is daar ook 'n onmiddelike toename in NO_3^- vlakke. Dit is as gevolg van die anorganiese vorm van die toegediende kunsmis. Die NO_3^- neem toe met tyd, terwyl die NH_4^+ vlakke afneem. Al die anorganiese N van kunsmis is onmiddelik beskikbaar, terwyl die N van slyk eers moet mineraliseer voor die anorganise fraksie vrykom en dit opneembaar vir plante is. Die NO_3^- produksie van slyk is gematig en konstant, en na 28 dae het die totale NO_3^- produksie van slyk die van kunsmis oortref. Selfs al produseer slyk meer NO_3^- as kunsmis, is die verspreiding daarvan meer egalig en kan dit meer effektief benut word deur plante. Die moontlikheid van NO_3^- loging by kommersiële kunsmis kan dus 'n groter logingsgevaar in terme van NO_3^- inhou as riool slyk.

'n Veldproef is gedoen om bogenoemde resultate met veldtoestande te vergelyk. Die potensiële loging en effek van plant opname was ook gemonitor. Twee proewe was gedoen: een vir winter en een vir somer toestande. Die proewe het vir drie maande aangehou en grondmonsters is twee-weekliks geneem op dieptes van 0-30 cm, 30-60 cm en 60-90 cm. Vir die winterproef is slyk van 4, 8 en 16 ton ha^{-1} toegedien. Mikrobiese aktiwiteit gedurende hierdie periode was laag, en gevolglik was ook lae NH_4^+ en NO_3^- vlakke gemeet. Geen loging het voorgekom nie. Vir die somerproef is die toedienings vlakke aangepas na 20 ton ha^{-1} slyk en ooreenkomstige kunsmis behandeling. Mielies is as landbougewas gebruik om die invloed van plante op die potensiële NO_3^- loging te bepaal. Geen betekenisvolle verskille was gevind tussen die oop blokke en gewas blokke nie. Betekenisvolle loging het voorgekom onder die kunsmis behandeling, maar geen loging is gemeet onder slyk behandelings nie. Mineralisasie is ook heelwat stadiger in die veldproef as in die laboratoruimproef, en na 90 dae was daar steeds NH_4^+ produksie in die boonste horison.

'n Gedeelte van die organiese N in slyk gaan verlore deur denitrifikasie. Hierdie verliese kan die beskikbare anorganiese N in die grond verlaag en gevolglik ook die potensiële risiko van NO_3^- loging. 'n Inkubasieproef is gedoen om die gas verliese van N deur N_2O en N_2 te bepaal. Gas monsters is geneem en geanaliseer met behulp van 'n gaschromatograaf. Toedieningsvlakke soortgelyk aan die van die vorige inkubasieproef is gebruik. Denitrifikasie was by twee verskillende voginhoude gemeet, 50 en 100% veldkapasiteit (VK). N_2O produksie van slyk verhoog onmiddelik na toediening, waarna die produksie tempo verlaag tot by dag 14. Geen betekenisvolle verskille is gevind tussen die 50% en 100% VK nie. Min tot geen N_2O produksie is gemeet vanaf die kunsmis toedienings.

Vanaf die data van die inkubasie proewe was N-kinetika gedoen. Hierdie waarde dui die tempo van slyk mineralisasie aan en die gevolglike tempo waarteen anorganiese fraksies (NH_4^+ en NO_3^-) vrykom. N-balanse was ook opgestel om die hoveelheid organise N wat gemineraliseer word, sowel as die veranderings tussen die verskillende anorganiese N fraksies, oor tyd aan dui.

Hierdie studie vergelyk die potentiële N besoedelik van rioolslyk met die van kommersiële kunsmis in landbougronde. Deur gebruik te maak van die bogenoemde resultate kan 'n beter begrip gevorm word oor die dinamika van rioolslyk in vergelyking met kunsmis. Hierdie kennis kan gebruik word om maksimum voordeel te trek uit die N inhoud van rioolslyk, sonder die negatiwe omgewingsimpakte soos bv. grondwater besoedeling.

CHAPTER 1

INTRODUCTION

1.1. BACKGROUND

It is common practice in South Africa to use agricultural land to dispose of sewage sludge. The disposal of sludge must however be done in a responsible manner and must meet the criteria originally outlined in: 'Guide: Sewage Sludge, Utilisation and Disposal' (Ekama, 1993)[1]. These guidelines were revised in 1997 to produce the new guideline: 'Permissible utilisation and disposal of sewage sludge, Edition 1' (WRC, 1997). Strict guidelines regarding sewage sludge disposal was formulated. The guideline deals exclusively with application of sewage sludge to land, and has been sited by the local water authority as the only acceptable criteria for sewage sludge disposal (Snyman *et al.*, 2000).

Snyman *et al.* (2000), discussed the non-compliance to the newly amended South African sludge guidelines and commented that:

i) The guidelines were revised, taking only the risk to the aquatic environment into consideration
ii) The ability of the wastewater treatment plants to generate these standards of sludge was not taken into account
iii) The guidelines in its present form discourages the beneficial use of sewage sludge as a soil ameliorant
iv) South Africa with its deteriorating soil conditions, cannot afford not to utilise the soil conditioning properties of wastewater sludge.

Any attempt to revise the latest guidelines to accommodate the limitations of available sludge handling technologies, the environment's ability to accumulate the potential pollutants and the risk of groundwater pollution will require more research (Snyman *et al.*, 2000). To gather more information concerning sludge application to agricultural land, a study group, organised by ERWAT (East Rand Water Care), was formed to study different aspects regarding sludge disposal, with particular attention to the beneficial application of sewage sludge to agricultural land.

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[1] References are listed at the end of Chapter 2

One major facet of concern that is emphasised in the newly amended sludge guidelines is the risk of NO_3^- leaching. There is, however, a need to investigate this aspect again to obtain a better understanding of the dynamics of N in sewage sludge and in soil. Once the behaviour of the sludge borne N is clearly understood, maximum beneficial use of sewage sludge as a N-fertilizer can be implemented.

The guidelines in SA are such, that the N content of the sludge determines the maximum annual application rates. Sludge application should be applied according to crop demand. However, in the guidelines, it was not taken into account that N in sludge exists predominant as an organically bound from. It must therefore first be transformed to inorganic forms by means of mineralisation prior to plant uptake. This means that the plant availability of N from sludge at any time is considerably lower than from commercial inorganic fertilizers. In certain situations the low rate of release of inorganic N from sludge could be a desirable feature, which may increase its availability. For example when the efficiency of N fertilisation is limited by factors such as high NO_3^- leaching losses, or high volatilisation losses of NH_4^+, the use of sludge as a slow release N material may decrease the N losses and increase N availability (Korentajer, 1991).

1.2. OBJECTIVES

The main objective of the study was to determine the extent of NO_3^- leaching as a results of sludge and fertilizer application in agricultural practises.

To achieve this objective, the following secondary objectives were pursued:

1. To compare the potential rates at which sludge is mineralised to produce NO_3^- and NH_4^+ to the availability of NO_3^- and NH_4^+ from commercial fertilizer;
2. To compare the quantities of NO_3^- that can leach from sewage sludge and commercial fertilizer treated soils under field conditions;
3. To determine the fraction of inorganic N losses through denitrification in sewage sludge and commercial fertilizer;

4. To calculate the rates at which N in sewage sludge is mineralised and nitrified to release inorganic N, in order to apply it to similar situation where sewage sludge is used as a fertilizer, in order to minimize the NO_3^- leaching risk and possible groundwater contamination;
5. To complete a balance statement on the different N fractions, during incubation of sewage sludge and commercial fertiliser treated soil respectively.

Each of these objectives is discussed in separate chapters in this thesis.

	Chapter 3	**Chapter 4**	**Chapter 5**	**Chapter 6**
NO_3 and NH_4 production	✓	✓		✓
Plant uptake and leaching		✓		
Denitrification			✓	✓
Mineralisation rates	✓			✓
N balance equation				✓

CHAPTER 2

LITERATURE REVIEW

2.1. INTRODUCTION

Wastewater treatment plants (WWTP) treat large volumes of domestic and industrial wastewater, and in the process produce sewage sludge as a by-product. Handling of this sludge is the most costly part of the sewage treatment, (Hughes *et al.*, 1993; Tchobanoglous & Burton, 1991) and tends to be an increasing problem, due to scarcity of dedicated land disposal sites and the potential of pollution. Previous methods of sludge disposal, such as ocean dumping and incineration, are restricted by law, because of the unsustainability of these practices (Kirkham, 1982; Korentajer, 1991; Hue *et al.*, 1988).

The advantage of applying sludge to agricultural land has been realised in resent years. It offers an economical and effective way of disposing sludge. It also has the environmental benefit of nutrient recycling (Lerch *et al.*, 1990). Sludge application to soil can improve certain soil properties such as soil fertility, soil organic matter content, microbial activities and residual nitrogen content. Physical properties can also be improved, such as an increase in porosity, decrease in bulk density, stabilisation of aggregates and soil structure and an increase in water holding capacity (Stamatiadis *et al.*, 1999; Hughes *et al.*, 1993; Metzget & Yaron, 1987). Sludge can increase the available macro- and micro-nutrients content in the soil, and thereby reduce the need to add commercial fertilizer (Environment Canada, 1984; Korentajer, 1991). Application of sewage sludge in the agricultural sector therefore has the advantage of improving the soil quality; increasing crop yields (Magdoff & Amadon, 1980), and reducing fertilizer costs, while at the same time disposing of sludge.

Unfortunately, the impact sludge has on the environment can also be negative. This forces operators of wastewater treatment plants to ensure that the disposal of sewage sludge is done in a sustainable way (Lötter & Pitman, 1997). The potential hazards associated with sewage sludge include accumulation of heavy metals, pathogens, toxic organics, and nitrate (NO_3^-) accumulation (Korentajer, 1991).

The potential NO_3^- content is often the limiting factor when disposing sludge. Worldwide, there is concern over increasing contamination of surface and ground water by NO_3^- due to agricultural practices and the potential threat to human and animal health (Brye *et al.*, 2001; Ross, 1989).

Guidelines exist in South Africa for sludge application in terms of total N application, because of the possibility of NO_3^- leaching. According to the 'Permissible Utilisation and Disposal of Sewage Sludge' (WRC, 1997), the maximum loading rate of sewage sludge to agricultural land is 8 tons sludge per hectare per year on a dry basis. No equivalent guidelines exist for commercial fertilizer. Such guidelines should be considered, because commercial fertilizer is a major source of N pollution. Unlike sludge, which contain some N in an organic form, most commercial fertilizers contains N only in the inorganic form. This means that N from fertilizer will be immediately available for plant uptake and leaching, whereas organic N from the sludge are only partially available and the rest must first be mineralised by microorganisms, before it can be utilised. In sludge, the plant available N is about 30% of the total N in the first year, 15% becomes available in the second year, and 5% in the third (WRC, 1997). This assures a more balanced and constant supply of N to the plant for a longer time than commercial fertilizers (Environment Canada, 1984). Commercial fertilizer can therefore potentially contribute more to groundwater contamination than sludge.

Given the significant amount of information concerning the potential harmful effects of sludge application, municipalities need information that will allow them to apply sludge, on an ongoing basis, at loading rates that will provide maximum benefits to agricultural practices, while minimising the environmental impact (Lerch *et al.*, 1990).

2.2. SLUDGE DISPOSAL

In the past, sludge was disposed by 1) ocean dumping, 2) dewatering and disposal in landfills, 3) incineration and 4) lagooning (Kirkham, 1982; Korentajer, 1991). These methods do not solve the problem of sludge disposal or pollution, it merely displaces it. More recently, the advantages of disposing domestic and industrial sewage sludge as a fertilizer in the agricultural sector has been realised, and became an acceptable way of discarding excess sludge. Application of sewage sludge to land is considered an inexpensive and environmentally acceptable method of sludge disposal. Simultaneously, it can act as a supplement of conventional inorganic fertilizer (Korentajer, 1991; Terry *et al.*, 1979). The positive aspects of using sewage sludge on the soil are numerous and to obtain these improvements, a large volume of sludge needs to be applied to the soil. Unfortunately, this can give rise to negative conditions associated with sludge application (Stamatiadis *et al.*, 1999). For sewage sludge to be disposed safely, it must be:

- environmentally acceptable;
- cost effective;
- minimise the environmental pollution;
- operationally feasible, and;
- politically/socially acceptable (Lötter & Pitman, 1997).

There is pressure on municipalities to use environmentally sound disposal practices. Due to increasing population growth and industrialisation, municipalities and industries in South Africa are generating increasing volumes of sludge. In evaluating the potential for increased sludge use in agriculture, careful consideration of the benefits of sludge applications and its potential health and environmental hazards is needed (Korentajer, 1991).

In South Africa, sludge is classified into four groups:

Type A:	Unstable sludge with an unpleasant odour; it contains a high level of pathogenic organisms. Primary/raw sludge falls into this group.

Type B	Stable sludge with a low and less offensive odour; it contains a reduced level of pathogenic organisms. Humus, waste activated and anaerobically digested sludges falls into this group.
Type C	Stable sludge with an insignificant odour; it contains an insignificant level of pathogenic organisms. Anaerobically digested sludge when preceded or followed by pasteurisation falls into this group.
Type D	Similar to type C; but it has a specified maximum concentration of metals and certain other elements (WRC, 1997; Ross *et al.,* 1992).

2.2.1. Composition of sludge

Wastewater consists of a mixture of organic and inorganic solids (Ross *et al.,* 1992; Fuller *et al.*, 1982). Subsequently, nutrients in sludge that is derived from wastewater can contain both compounds of agricultural value and pollutants (European Commission, 2002). The composition of sludge is extremely variable (Korentajer, 1991), but it does contain significant quantities of essential plant nutrients like N, P and K, as well as other nutrients such as Ca, Mg, Fe, S, etc. Potential pollutants include Cu, Cr, Ni, Zn and Cd (Ross *et al.,* 1992; European Commission, 2002), which can become toxic if they exceed certain concentrations. Organic C, total P and S tend to be relatively constant in different types of sludges. Most sludges contain 20-30% (m/m) organic C, and 1-4% (m/m) inorganic C (Sommers, 1977). Sewage sludge proteins are significant sources of labile C and their degradation products are an important N source (Bernal *et al.*, 1998). Total N is more variable and values can range from 2-4% (m/m) (Sommers, 1977), depending on factors such as the origin of the sludge and climatic conditions (Korentajer, 1991). NO_3^- will probably not be present in 'fresh' sludge, but will form over time through the process of nitrification (Sommers, 1977).

The characteristics of sludge depend on the original pollution load of the treated water, the technical characteristics of the wastewater and the sludge treatments carried out (European Commission, 2002). Sludge that is derived from domestic wastewater contains trace elements in the suitable ratios to suit the requirements for most crops.

In the case of industrial wastewater, the heavy metal content in the sludge is higher, and could cause metal accumulation in the soil (Ross *et al.*, 1992).

The average composition of 77 South African wastewater treatment plants is presented in Table 2.1 (Smith & Vasiloudis, 1989).

Table 2.1: Sludge characteristics of 77 South African wastewater treatment plants

Parameter	**Concentration** (mg kg^{-1})	**Standard deviation** (mg kg^{-1})*
Total Nitrogen	31070	9780
Total Phosphorus	15570	7960
Potassium	2550	1990
Cadmium	13	25
Chromium	551	1206
Copper	655	1945
Lead	455	1151
Nickel	155	342
Zinc	2054	2176
Mercury	5	4
Arsenic	7	5
Selenium	3	12
Molybdenum	7	4
Boron	30	14
Fluoride	128	152

* Dry mass base

2.2.2. Advantages of sludge application to soil

It is well known that the application of organic material can improve the physical and the chemical properties of a soil. These beneficial effects are even more noticeable in soil with low organic matter content.

i. Chemical improvements

There is a substantial quantity of nutrients in sludge, such as N, P, K and Ca (Hue, 1988), although the concentrations of N, P and K are lower than in inorganic fertilizers. A major beneficial characteristic of the sludge is that these nutrients are in the organic form and needs to be mineralised before it becomes mobile and this assures a more constant supply of the nutrients in the soil (Ross *et al.,* 1992). According to Hue (1988), the effect of sewage sludge on the soil is still evident up to three years after application. Increases in crop yields can be observed from sludge treated plots (Fuller *et al.*, 1982; Hue, 1988). Applying sludge as a source of major elements may simultaneously correct micronutrient deficiencies, such as Cu, Mn, Zn, Mo and B. It was found that sludge was particularly effective to supply Fe and Zn (Korentajer, 1991).

Application of sewage sludge to acid soils can increase the soil pH, on the surface as well as in the subsoil (Hue, 1988; Fuller *et al.,* 1982).

ii. Physical improvements

The gradual decrease in the organic matter content in cultivated soils is of particularly concern in warm areas, where organic matter decomposes rapidly (Metzger & Yaron, 1987). By applying sludge, the organic status of the soil can be increased, and the physical properties of agricultural land improved (Tester *et al.*, 1977). The organic matter in sludge can improve soil structure and reduce runoff and erosion (Korentajer, 1991).

Sludge can increase the water retention of a soil due to the higher water retention of organic matter, and more indirectly by improving other physical soil properties (Metzger & Yaron, 1987). According to Wei *et al.* (1985), water retention improves only significantly at high application rates, such as 112.0 to 134.4 ton ha^{-1}.

Application of sludge can reduce the bulk density of a soil and studies have shown that three years after sludge incorporation, significant reductions in bulk density were still found, even at low application rates of 27 ton ha^{-1} (Metzger & Yaron, 1987). This will naturally depend on the soil type.

2.2.3. Disadvantages of sludge in the soil

Sewage sludge contain a number of pollutants, or potential pollutants, such as NO_3^-, heavy metals, toxic chemical and pathogenic organisms that may endanger human and animal health and impair the growth of plants.

i. NO_3^- leaching

NO_3^- can pose cause environmental pollution if it is not controlled (Gaines & Gaines, 1994), and therefore the N content is often the primary factor that limits sludge application (Higgins, 1984; Douglas & Magdoff, 1991). If applied in large volumes, excess N can cause undesirable changes in soil properties, such as a decrease in pH due to nitrification. Accumulation of NO_3^- in the soil can lead to luxury consumption by plants (Magdoff & Amadon, 1980; Artiola, 1991) and leaching can create a potential for groundwater contamination (Stamatiadis *et al.*, 1999; Artiola, 1991).

N is leached mainly as NO_3^-, but in sandy soils NH_4^+ can be leached as well. Unlike heavy metals and toxic organics, NO_3^- is not adsorbed in the soil constituents and is therefore very mobile in the soil. This is a particularly serious problem in areas with shallow groundwater tables (Korentajer, 1991). Leaching of N is common where soil NO_3^- levels are high, and where excess rain or irrigation can cause downward movement in the soil. These soil conditions are largely found in humid and sub humid regions (Stevenson, 1986). Fluctuations of NO_3^- concentrations in soil water occur throughout the year. This can be due to a low field capacity, differences in precipitation and plant uptake, etc (Hansen & Djurhuus, 1997).

If sludge is applied to crops, the uptake by plants itself and water uptake of plants could reduce NO_3^- leaching, especially in a dry country like South Africa. This is not taken into account in the existing guidelines.

Some physical soil properties, such as texture and structure, can influence NO_3^- leaching, because it has an influence on the water infiltration, permeability and water holding capacity of a soil (Environment Canada, 1984). It can therefore be expected that sandy soil, which has lower water holding capacity and high infiltration rate, will have a higher NO_3^- leaching potential than clayey soils (Gaines & Gaines, 1994).

Higgins (1984) found that NO_3^- increases in the lower horizons are only detectible a year after sludge application. NO_3^- does not accumulate in the soil, and is easily removed by leaching or denitrification. The effect of sludge application on groundwater in terms of NO_3^- is temporary and easily recoverable (Higgins, 1984).

ii. Heavy metals

High concentrations of heavy metals in some sludge, especially sludge from WWTP that receive industrial effluents, can reverse the benefits to soil fertility and plant nutrition. Heavy metals such as Cd, Ni or Pb, Hg, Zn and Mo can especially become problematic if it accumulates in the environment to become a long-term pollution problem (Korentajer, 1991; Hue, 1988, Lerch, *et al.*, 1990). Cu, Zn and Ni can become phytotoxic (toxic to plants) (Korentajer, 1991; Higgins, 1984) and reduce yields or lower the quality of food of fibre produced (Sommers, 1977). Cd, Pb, Hg and As are toxic to humans, of which Cd appears to be the greatest threat (Higgins, 1984), because of its relatively high mobility in the soil (Korentajer, 1991).

iii. Toxic organics

Numerous organic compounds are present in sludge, which can, after application, be physically, chemically and biologically transformed into other compounds by means of mineralisation. The degradation of organic compounds is thus greatly dependent on the aerobic of anaerobic conditions. Most organic pollutants are not taken up by plants, and can only be a risk if sludge is applied directly onto crops (European Commission, 2002).

iv. Pathogenic organisms

Pathogenic organisms can also be present in sludge, and this can be potentially dangerous to human health (Ross *et al,* 1992; Lerch *et al.*, 1990; Higgins, 1984). There are five main types of pathogens in sludge, such as bacteria, viruses, fungi and yeasts, parasitic worms and protozoa to which humans and animals can be sensitive too. These pathogens are mainly present on the soil surface or at shallow depths, where sludge has been incorporated into the soil (European Commission, 2002). The potential problem of biological pollution by sewage sludge is the contamination of surface water and ground water by pathogens transported by runoff and percolation water (Korentajer, 1991). Even so, the majority of pathogens in sludge could rapidly be inactivated in the soil system through sunlight and competing soil microorganisms (Snyman *et al.*, 1998).

2.3. THE CHEMICAL TRANSFORMATION OF SOIL N

The dynamics of N in soil systems are well described in literature and this knowledge can also be applied to soils enriched with sewage sludge.

2.3.1. Forms of N present in soil

Under normal conditions the total amount of N in the soil consists mainly of organic N, NH_3, NO_3^-, and NO_2^- (Tchobanoglous & Burton, 1991, Mengel & Kirkby, 2001).

i. Organic N

The biggest pools of N in the plant-soil system are present as organic N in plants, humus and litter (Singer & Munns, 1992). The organic N compounds have a very low solubility (Sveda *et al.*, 1992), and are unavailable for plant uptake in this form (Follett *et al.*, 1981).

ii. Ammonia (NH_3) and ammonium (NH_4^+)

In soils NH_3 will be dissolved in the soil solution and depending on the pH, it occurs as either an ammonium ion (NH_4^+) or ammonia (NH_3).

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

In cases where the pH is above 7, the equilibrium will be shifted to the left, and at a pH of below 7 the equilibrium shifts to the right and the NH_4^+ ion is predominant (Tchobanoglous & Burton, 1991).

Under anaerobic soil conditions the NH_4^+-content can be high, but is usually low under aerobic soil conditions because of fast nitrification of NH_4^+ (Mengel & Kirkby, 2001). In most soils NH_4^+ will be absorbed, but in soils with a very low cation exchange capacity, the NH_4^+ can leach in significant quantities (Ross, 1989).

iii. *Nitrite, NO_2^-*

Under normal conditions NO_2^- seldom exceeds a level of 1 mg L^{-1} in surface water or ground water (Van Cleemput & Samater, 1996; Tchobanoglous & Burton, 1991). In spite of its low concentrations it is extremely toxic to most fish and other aquatic species (Tchobanoglous & Burton, 1991). As it is unstable it can transform to gaseous N compounds and contribute to acid rain and the greenhouse effect (Van Cleemput & Samater, 1996).

Under anaerobic conditions NO_2^- is relatively unstable, and transforms easily into NO and NO_3^- (Tchobanoglous & Burton, 1991). It is an intermediary compound during denitrification and nitrification processes. Accumulation of NO_2^- in soils has been reported to occur during nitrification after urea applications. NO_2^- is to some extent stable under alkaline conditions, and can accumulate under high pH conditions and is toxic to plants (Samater & Van Cleemput, 1996).

iv. *Nitrate, NO_3^-*

NO_3^- is the most oxidised form of N and can be utilised by plants (Tchobanoglous & Burton, 1991). The concentration of NO_3^- in the soil solution may change quickly due to uptake by plant roots and microorganisms, leaching and denitrification. Conditions favouring nitrification include high temperatures and well-aerated soils (Mengel & Kirkby, 2001).

NO_3^- losses also depend on fertilizer source, plant species, soil type, soil structure and texture, climatic conditions and management practices. In humid areas NO_3^- can almost completely be removed from the soil profile by leaching, denitrification or both (Sveda *et al.*, 1992).

In cultivated lands a major source of NO_3^- can be the nitrification by microorganisms when N is applied in the reduced form. Oxidation of NH_4^+ produces HNO_3 and is an important cause of long-term soil acidification in agriculture (Singer & Munns, 1992).

2.3.2. N cycle

The N in the soil accounts for an important part of the complete N cycle in nature (Stevenson, 1986). A major part of the N in the N cycle, is the N_2 in the atmosphere that is made up of 79.08% N_2 by volume (Stevenson, 1986; Singer & Munns, 1992). All forms of N in the soil solution are affected by a number of dynamic equilibrium reactions such as sorption, desorption, precipitation, dissolution, immobilisation, mineralisation, nitrification, denitrification, volatilisation and leaching (Van Cleemput & Boeckx, 1999). Because N is a very mobile element circulating between the atmosphere, the soil and living organisms, a N cycle does not really exit as such in nature. Any given N atom just moves from one form to another in an irregular manner. Furthermore, the soil has an internal cycle that is distinct from the overall N cycle, but has interfaces with it. This internal cycle is characterised by mineralisation - immobilisation (Mengel & Kirkby, 2001; Stevenson, 1986). The main steps of N transformation in the soil are mineralisation, immobilisation, nitrification, denitrification, ammonia volatilisation and leaching cycle (Figure 2.1) (Environment Canada, 1984).

The following N cycle has been outlined by Owen & Chiras (1995):

1. N diffuses form the atmosphere into air spaces of the soil;
2. N fixing bacteria combine N_2 gas and H_2 to form NH_3 that is utilised by plants and other bacteria;
3. Plants are consumed by animals, which requires N for protein synthesis;
4. Some N can return to the soil in the form of excreta and urine or carcasses of dead animals;
5. The large organic compounds containing N from animals or plants are mineralised by bacteria and transformed to NO_3^- in the process of nitrification;
6. NO_3^- can be utilised by plants and the cycle can start over again;
7. Some N can go back to the atmosphere and this is accomplished by denitrifying bacteria in soil.

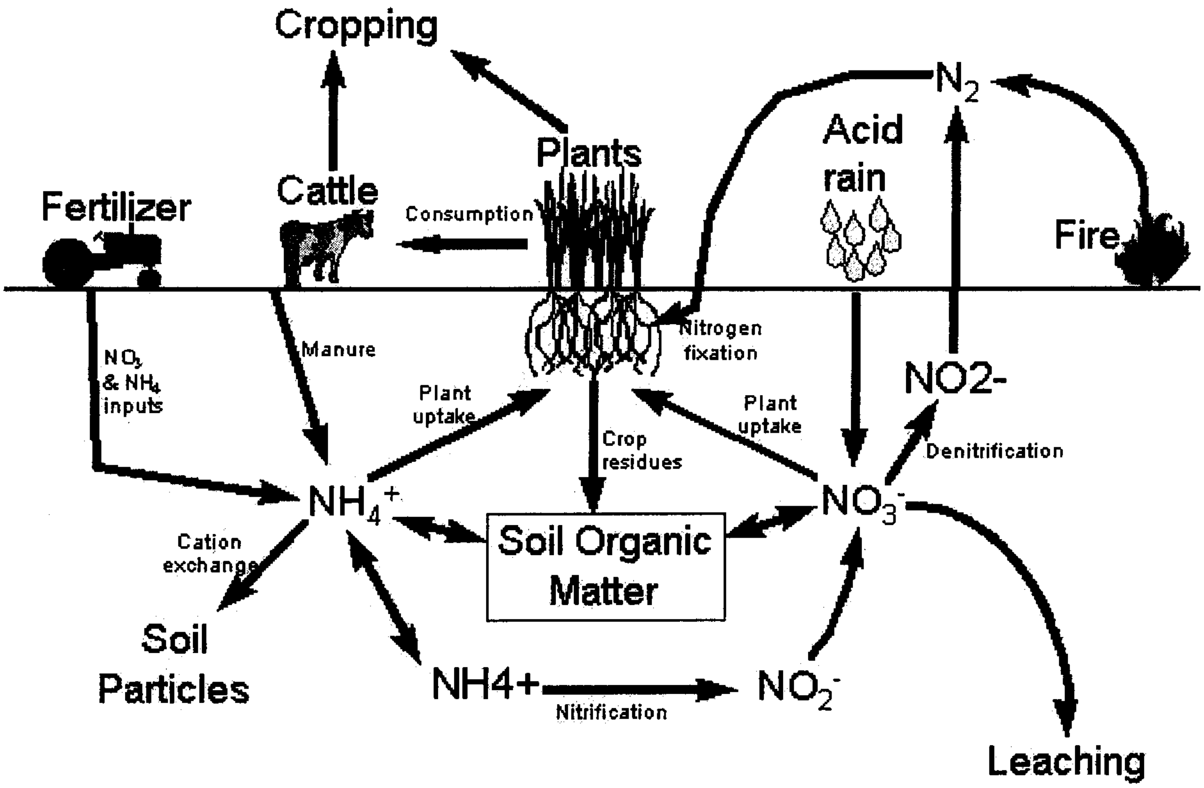


Figure 2.1: Schematic presentation of the N cycle

2.3.3. N processes

The most important processes through which the N cycle in the soil takes place, are summarised in Table 2.2.

Table 2.2: Processes of the N cycle, as summarised by Ross (1989)

Type of process	Process description
Storage	Chemical fixation of NH_4^+ by cation exchange on negatively charged soil colloids
Transformation	i. Ammonification: mineralisation of organic N by a wide range of decomposer organisms ii. Nitrification: Bacterial transformation of NH_4^+ to NO_2^- and then NO_3^- iii. Denitrification: reduction of NO_3^- to NO_2^- followed by further reduction to the gases N_2O, NO and N_2
Transport	i. NO_3^- leaching: NO_3^- is very soluble and subject to mass flow and drainage out of soil profiles during water percolation ii. Volatilisation: a) N_2O, NO, N_2 after denitrification b) NH_3 losses from alkaline soils

The important processes concerning N transformation in the soil that are associated with sludge application, also mentioned in Table 2.2, will shortly be discussed.

i. Mineralisation and immobilisation

Mineralisation is the transformation of an element from an organic to an inorganic state as a result of microbial action (Van der Watt & Van Rooyen, 1995). Microbial degradation of organic components is central in the ultimate fate of sludge in soils (Terry *et al.*, 1979). Soil organisms are classified as autotrophic or heterotrophic on the basis of their C and energy sources. Heterotrophic organisms obtain their C from breakdown of organic material. Nearly all the heterotrophic organisms obtain their energy form the oxidation of the carbon in organic compounds. This group is the largest group of organisms in the soil, and is responsible for organic decay. Autotrophs obtain their carbon from CO_2 or carbonate minerals and their energy from different sources, like sunlight, or oxidation of inorganic materials, such as N, S and Fe (Brady & Weil, 1999; Alexander, 1977).

Mineralisation of N from organic molecules starts with hydrolyses by microorganisms, that leads to the formation of NH_3 (Mengel & Kirkby, 2001). The N content of organic material has a major effect on microbial activity and the subsequent degradation of organic matter, such as sludge (Stevenson, 1986; Metzger & Yaron, 1987). In this process part of the C and N is converted into microbial tissue, and part is released as inorganic N and CO_2 (Janssen, 1996). In soil the microorganisms compete with higher plants for the mineralised N. This N that is taken up by heterotrophic organisms will only be released as NH_4^+ when they die (Alexander, 1977).

Immobilisation occurs when organic matter has a low N content that results in mineralisation rates that are too low to satisfy the needs of the microorganisms. The net effect of mobilisation and immobilisation depends on how well the sludge provides N to the microorganisms. If sludge has a high N content, some N will become available to plants, but if it provides insufficient N, microorganisms will absorb most of the NH_4^+ for their own requirements, resulting in N shortages (Singer & Munns, 1992; Janssen, 1996). Immobilisation by soil microorganisms may temporarily keep NO_3^- from leaching, but the immobilised N will eventually be mineralised and is available for leaching after it is nitrified (Lowrance & Smittle, 1998).

a. Factors determining mineralisation rate

Mineralisation of organic matter is highly variable and depends on a range of different variables (Leiros *et al.*, 1999; Korentajer, 1991). The major factors that influence mineralisation are moisture, temperature and sludge composition, which controls the mineralisation processes through their effects on microbial activity in the soil (Leiros *et al.*, 1999; Metzger & Yaron, 1987). Soil moisture affects the decomposition of organic carbon from sludge in two ways. At low moisture content, the water supply for the growth of microorganisms is insufficient while at high water content the oxygen supply becomes limiting. The optimum water content for microbial activity is at field capacity (Metzger & Yaron, 1987). The optimum temperature for mineralisation is between 30 and 35°C. Increases in temperature, lead to an increase in decomposition of sludge (Metzger & Yaron, 1987; Stanford & Smith, 1972).

Besides the microbial activity, the extent of mineralisation of sludge is strongly affected by the type of sludge utilised (Hernandez-Apaolaza, 2000) or, more specifically, the type of treatment to which sludge is subjected before application. Soil parameters (e.g. texture, pH), environmental conditions (temperature, moisture) and application conditions (loading rate, method) can also influence the decomposition rate of the sludge (Metzger & Yaron, 1987; Greyling *et al.*, 1990; Korentajer, 1991; Leiros, *et al.*, 1999; Trindade, *et al.*, 2001; Higgins, 1984).

b. C:N ratio

An important parameter to predict the mineralisation rate of applied sludge is its C:N ratio (Singer & Munns, 1992). If the N content of applied organic material is very high, the C:N ratio will be low (<20). In this case the microflora can satisfy their needs from the N source, and the surplus is released as NH_4^+. If the applied organic material has a low N value, (high C:N ratio, >20) the decomposition of organic material is slow (Singer & Munns, 1992; Tester *et al.*, 1977).

ii. Ammonification

The conversion of organic N into NH_3 by microorganisms is called ammonification.

iii. Nitrification

Nitrification is the process where NH_4^+ is oxidised to NO_3^-, with NO_2^- as transition product (Laubcher & Du Preez, 1991). This is a complex process that is influenced by physical, chemical and biological factors. NH_3 is first oxidised to NO_2^-, then to hydroxylamine (NH_2OH) and finally to NO_3^- (Mengel & Kirby, 2001).

$$NH_3 + O_2 \rightarrow NH_2(OH) + \tfrac{1}{2}\,O_2$$

$$NH_2(OH) + H_2O \rightarrow HNO_2 + 4e^- + 4H^+$$

Nitrite oxidation follows the equation:

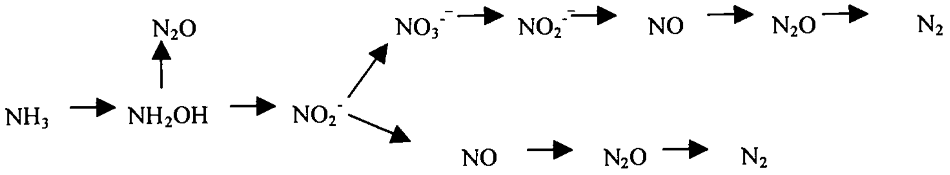
$$HNO_2 + H_2O \rightarrow HNO_3 + 2e^- + 2H^+$$

Two specialised groups of bacteria are involved, *Nitrosomonas,* a subgroup autotrophic organisms, will utilise the NH_4^+ and oxidise it to NO_2^-, followed by another subgroup, *Nitrobacter*, which oxidises the NO_2^- to NO_3^- (Alexander, 1977). Both NH_4^+ and NO_2^- oxidisers are aerobic and in most habitats they are closely associated so that the NO_2^- produced is rapidly oxidised and does not accumulate. In waterlogged soils, the oxidation of NH_4^+ is restricted because of a lack of O_2 required for the oxidation. The nitrifying bacteria prefer neutral soils rather than acid soils, and nitrification is usually depressed in acid soils (Mengel & Kirby, 2001; Tester *et al.*, 1977), but is dominant at high pH (Vermoesen *et al.*, 1996). The optimum rate of nitrification occurs at a pH of 8.3-8.5 (Follett *et al.*, 1981).

As a result of the conversion of NH_4^+ to NO_3^-, the pH of the soil lowers (Stevenson, 1986; Singer & Munns, 1992) and both oxidation reactions lead to the formation of acids: NH_3 forms nitrous acid (HNO_2), and HNO_2 forms nitric acid (HNO_3) upon oxidation. Acid formation is the result of the release of H^+ ions in the soil solution. The formation and accumulation of the strong nitric acid is the cause of acidification of soils during nitrification (Mengel & Kirkby, 2001; Singer & Munns, 1992).

iv. Denitrification

Denitrification is the reduction of NO_3^- and/or NO_2^- by bacteria, to gaseous compounds, NO, N_2O and N_2 (Freney & Simpson, 1983; Singer & Munns, 1992; Stevenson, 1986). Only a few organisms have the ability reduce NO_3^- and favourable conditions for denitrification is: anaerobic conditions where the oxygen concentration is very low, a warm environment, abundance of organic matter and NO_3^-, and low soil pH (Mitchell *et al.*, 2000; Singer & Munns, 1992; Van Cleemput & Samater, 1996; Brye *et al.*, 2001). At several places in the N-cycle N_2O as well as NO_x (NO+ NO_2) can be produced. The applicable reactions are given below:



Denitrifying bacteria use the oxygen of NO_3^- (and NO_2^-) as an electron acceptor instead of O_2 (Stevenson, 1986). From a crop production point of view, denitrifying bacteria usually play a negative role by volatilising the NO_3^- and thus causing a loss of plant available N. From an environmental quality perspective, denitrification can be both positive and negative: positively, it can reduce the available NO_3^- and decrease the risk of groundwater pollution (Lowrance & Smittle, 1988; Singer & Munns, 1992); negatively it can contribute to the greenhouse effect through N_2O production (Vermoesen *et al.*, 1996). Denitrification can be responsible for up to 30% of total loss of N from agricultural soils (Singer & Munns, 1992), and nearly 70% of the anthropogenic emissions of nitrous oxides (N_2O) to the atmosphere (Brye *et al.*, 2001).

v. Ammonia Volatilisation

NH_3 is a gas at normal atmospheric temperatures and pressures and it can be expected that NH_3 will volatilise to the atmosphere if present in the soil. It is however a basic gas that easily reacts with protons, metals and acidic compounds. It also has a strong affinity for water and forms NH_4OH in the soil solution (Freney & Simpson, 1983).

Different quantities of NH_3 can be lost from the soil and this loss varies from as little as 3% to as much as 50% (Stevenson, 1986). These losses will depend on soil properties (clay content, CEC, pH), plant-uptake, environmental factors (temperature, moisture, atmospheric concentration of NH_3) and farming practices (Freney & Simpson, 1983). When fertilizer is incorporated in the soil, the losses due to volatilisation will be small, especially if the soil is acid or neutral. NH_3 losses can be significant if the fertilizer is applied on the soil surface of alkaline or calcareous soils or when the soil is dry (Stevenson, 1986; Van Cleemput & Boeckx, 1999).

vi. Plant uptake

Most crops can utilise N in the form of NO_3^- and for agricultural crops it may be the most important source of N that is available in the soil solution (Stevenson, 1986). . Because NO_3^- is an anion and susceptible to leaching, the use by plants can reduce NO_3^- leaching to the ground water (Hansen & Djurhuus, 1997).

vii. Erosion and runoff

Because of the high NO_3^- content of the topsoil, large quantities of N can be lost by soil erosion and surface runoff of water (Stevenson, 1986).

2.3.4. Environmental hazards of N

There are several environmental hazards associated with N, such as health risks, pollution of aquatic systems by NO_3^-, or the contribution to greenhouse gases by gaseous N emissions.

i. Health hazards

NO_3^- becomes toxic to animal and humans, especially infants, if consumed in large concentrations. NO_3^- is associated with methemoglobinemia, or 'blue baby syndrome'. Infants and young animals can suffer from this, and ultimately die, but healthy older animals and adults have stomach acids that make this poisoning unlikely (Ross, 1989; Sveda *et al.*, 1992; Miller & Gardiner, 1998). If sub-lethal concentrations are consumed by ruminant animals, it can lead to a reduction in milk production, abortions, and metabolic disorders (Die Kynoch Weidings-handleiding, 1997).

ii. Groundwater contamination

NO_3^- leaching to groundwater is considered to be a major problem in the agricultural sector. One of the major contributors to groundwater pollution by NO_3^- is the use of more N than is actually needed by crops (Magdoff, 1992). NO_3^- can result in eutrophication of watercourses and algal blooms (Ross, 1989).

Because of the risks associated with NO_3^- pollution, there are strict specifications regarding NO_3^- content in drinking water (Sveda *et al.*, 1992). Drinking water standards in South Africa are 10 mg L^{-1} NO_3^--N, as legislated by the Department of National Health and Population Development (Korentajer, 1991). The levels of NO_3^--N in wastewater usually vary from 0-20 mg L^{-1}, with the maximum at 45mg L^{-1} (Tchobanoglous & Burton, 1991).

Overseas norms for sludge N application rates may not be applicable to the local conditions. Additional research is required to assess the rate of mineralisation and crop N uptake to ascertain the dangers of NO_3^- leaching and accumulation in plants under South African soil and climatic conditions (Korentajer, 1991).

iii. Greenhouse gases

Many authors predict that over the next century the global temperature will increase between 1.4 and 4°C (Leiros *et al.*, 1999). The major contributor to greenhouse gases is the burning of fossil fuels, such as petroleum, coal, and natural gas. Recently the contribution of agricultural practices is being recognised more and more. In the process of clearing land and forests to introduce new land for cultivation, plant material is burned, while other areas are flooded. Ruminant animals are introduced to the field and application of N fertilizers is all activities that contribute to the emission of greenhouse gasses (Hillel, 1998).

Gasses that are mainly emitted are CO_2, CH_4, and N_2O. In total agricultural sources contribute to about 20% of the anthropogenic emissions of greenhouse gasses. N_2O occurs in the atmosphere in much smaller concentrations, but have been increasing over the last decades. The annual rate of increase of is 0.2-0.3% (Hillel, 1998), and the concentration has been estimated to have increased with 50% as compared to pre-industrial times. NO emission from soils seems to contribute significantly to global emissions (Vermoesen *et al.*, 1996). The production of N_2O in the soil is an intermediate stage in both nitrification and denitrification. It is during these processes that N is released from the soil to the atmosphere (Hillel, 1998; Vermoesen *et al.*, 1996).

2.4. SLUDGE AS A FERTILIZER

Studies have shown that the yield of many plant species increases following the application of sewage sludge; this increase can be attributed mostly to the increased supply of plant nutrients. In comparison with most commercial inorganic fertilizers, the concentration of plant nutrients in sludge is relatively low (Korentajer, 1991). The response of plants to sludge application depends on many factors, such as sludge type, composition, soil properties, climatic conditions, application level and method (Metzger & Yaron, 1987).

The use of sludge as a fertilizer has certain limitations, such as the high transport cost and the potential soil and ground water pollution that it may cause (Korantajer, 1991).

2.4.1. Sludge as a soil ameliorant

A gradual decrease in the organic matter content of cultivated soils all over the world becomes an increasing problem. In warm areas like Southern Africa the rate of organic matter decomposition by microorganisms is high (Korentajer, 1991; Metzger & Yaron, 1987). The decrease of organic matter may lead to a deterioration of the soil physical properties, that may in turn lead to accelerated erosion. The irreplaceable loss of agricultural soil due to soil erosion and the subsequent decrease in crop productivity are considered some of the main environmental problems on the African continent. The addition of sludge could provide a measure to maintain the organic status of soil and decrease the danger of runoff and erosion (Korentajer, 1991).

According to Act 36 of 1947, in South Africa a fertilizer can only be classified as an organic fertilizer if it contains less than 20% ash on a dry mass base and 40% water. Sludge does not comply with these criteria and can therefore not be classified as an organic fertilizer. Although it cannot be sold as a organic fertilizer, it can be used as a ameliorant if it meets certain criteria.

2.4.2. Sludge as a N-fertilizer

The N in sludge is mainly present in the organic form that must first be mineralised before it can be available to plants (Singer & Munns, 1992; Trindade, *et al.*, 2001).

Municipal wastewater and sludge contain variable amounts of organic N, NO_3^-, NO_2^-, and NH_4^+ (Environment Canada, 1984.). The quantity of N that can be applied to soil depends on the type of crop, the composition of sludge added to the soil, the rainfall etc. When the application rate of plant-available NH_4^+ exceeds the rate of N uptake by the plants, the surplus NH_4^+ will be oxidised by autotrophic bacteria to NO_3^-. Because NO_3^- is soluble in water, it can move down in the soil profile and contaminate the ground water (Ross *et al.,* 1992).

According to the guidelines: Permissible Utilisation and Disposal of Sewage Sludge (WRC, 1997), the total quantity of N in sewage sludge which can become plant available is about 30% in the first year of application, 15% in the second year and 5% in the third year. In a study done by Cripps *et al.* (1992) it was found that the mineralisation rate of N is 50% in the first year, and 30% in the second year. These rates will depend on the climatic conditions, soil and sludge type (Cripps *et al.*, 1992).

The guidelines in SA are such, that the N content of the sludge determines the maximum annual application rates. Sludge application should be applied according to crop demand. However, in the guidelines, it was not taken into account that N in sludge exists predominant as an organically bound from. It must therefore first be transformed to inorganic forms by means of mineralisation prior to plant uptake. This means that the plant availability of N from sludge at any time is considerably lower than from commercial inorganic fertilizers. In certain situations the low rate of release of inorganic N from sludge could be a desirable feature, which may increase its availability. For example when the efficiency of N fertilisation is limited by factors such as high NO_3^- leaching losses, or high volatilisation losses of NH_4^+, the use of sludge as a slow release N material may decrease the N losses and increase N availability (Korentajer, 1991).

Although the initial total concentration of N in the applied sludge is high, a large quantity can be lost through volatilisation (Cripps *et al.*, 1992). According to Korentajer (1991), the fraction of NH_3 volatilised from sludge can vary between 0% to 50%, depending on the form of sludge applied, method of application, and climatic factors.

To be able to manage a N fertilizer programme, the combined use of inorganic and organic matter inputs has been recommended as a means of maintaining high crop productivity and stable yields (Fernandes *et al.*, 1997; Geiger *et al.*, 1992).

2.5. COMMERCIAL FERTILIZER

N fertilizer application has important effects in terms of increasing crop production, because of the low natural N content in the soil (Mengel & Kirkby, 2001). The sources of N (NO_3^- and NH_4^+) fertilizer differ in effectiveness. Worldwide the use of inorganic fertilizer has increased tremendously between 1950 and 1984 and this has increased crop production. Without fertilizers the world food output would have dropped an estimated 40% (Miller, 1996; Cunningham & Saigo, 2001). The use of commercial inorganic N fertilizer simplifies the management of a N-fertilizer programme, in terms of quantity to apply and methods of application (Kowalenko & Cameron, 1978).

In general NO_3^- are used in larger quantities, but the effectiveness is not very high because it is subject to denitrification and leaching losses. NH_4^+ losses due to volatilisation may also occur, but to a lesser extent (Sveda *et al.*, 1992). Under cultivation, commercial fertilizers have the disadvantage that they do not add organic matter to the soil. This can lead to a decrease in soil organic matter, and subsequently in a decrease in the beneficial effect it has on the physical properties of the soil. Commercial fertilizer:

- decreases water holding capacity;
- decreases soil porosity;

- increases soil compaction; and
- lowers the o_2 content of the soil.

In addition, most commercial fertilizers supply only two or three of the essential nutrients needed by plants. Production, transport and application of commercial fertilizers also require a lot of energy and can have significant environmental impacts (Miller, 1996).

Inorganic N-fertilizers that is applied in the reduced form, such as NH_4^+ are acid forming, due to microbial oxidation of NH_4^+ to NO_3^- (Miller & Gardiner, 1998).

$$NH_4^+ + 2O_2 \xrightarrow{bacteria} NO_3^- + 2H^+ + H_2O$$

Commercial production of fertilizers requires energy, that is obtained from fossil fuels such as coal (Owen & Chiras, 1995; MVSA, 1997). By burning fossil fuels to manufacture N fertilizers, thee times the N-oxides and gaseous NH_3 are released into the atmosphere, compared to the natural processes in the N cycle (Miller, 1996). The initial product, NO, is oxidised further in the atmosphere to NO_2. The combination of NO and NO_2 is referred to as NO_x. NO_x combines with water in the atmosphere to form nitric acid (HNO_3), that returns to the earth in the form of acid rain, (Cunningham & Saigo, 2001; Miller, 1996).

Over-fertilisation often occur, because farmers may not be aware of the nutrient status of their soil and NO_3^- levels in groundwater increases as a result of this (Cunningham & Saigo, 2001; Ross, 1989). Apart from economical factors, the over-utilisation of commercial fertilizers can also have a negative effect on the environment. This definitely stresses the need to increase the use of organic matter (Fernandes *et al.*, 1997).

2.6. CONCLUSION

Large volumes of sewage sludge are produced each year. Disposing of this sludge as a fertilizer could be potentially harmful, but if applied correctly, it can improve the soil quality, and in some cases be more advantageous than commercial fertilizer. The existing guidelines of sludge disposal in South Africa, only allows 8 ton ha^{-1} year^{-1}. Higher application levels could be more beneficial to plants and improve soil qualities. Excessively large application rates on the other hand, can result in environmental pollution such as NO_3^- leaching. Maximum application rates of sludge will vary with soil type, climatic conditions, type of crop, etc.

In the following chapters, the application rate of 8 ton ha^{-1} year^{-1} will be re-evaluated and compared with an inorganic, commercial fertilizer. Different experiments were done to determine the maximum sludge application rates that would result in maximum benefits for plants, without the potential risk of NO_3^- leaching.

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CHAPTER 3

MINERALISATION AND NITRIFICATION RATES OF N UNDER SOUTH AFRICAN CONDITIONS

3.1. INTRODUCTION

N losses in agricultural systems can be an economic and environmental problem. These losses can take place through crop removal, leaching, surface run-off, NH_3 volatilisation, denitrification and erosion (Jarvis *et al.*, 1996). Loss of N from agricultural soils is of concern due to the possible NO_3^- leaching which is responsible for increasing concentrations of NO_3^- in surface and ground waters (Cheshire *et al.*, 1999).

N that is lost from the soil system can be restored by adding N-fertilizers, either in the inorganic or organic form. One such an N-source can be sewage sludge. Mineralisation of organic N is an important consideration when determining the appropriate rates for sludge application to cropland (Parker & Sommers, 1983). The processes of mineralisation and immobilisation are central to the control of the N flows within agricultural cycles (Jarvis *et al.*, 1996). Mineralisation of sewage sludge depends on the sludge characteristics (nature and quantity), environmental factors (temperature and humidity), and soil conditions (pH, accessibility for soil fauna, soil texture and structure), (Leiros *et al.*, 1999; Janssen, 1996; Parker & Sommers, 1983; Trindade *et al.*, 2001).

In contrast to the inorganic N that is immediately available to plants when applied, and easy to quantify, the N supply rate from organic forms depends on the mineralisation rates that are difficult to quantify. Quantification of organic N mineralisation rates is the first step necessary to improve the N use efficiency and to reduce the losses to the environment (Trindade *et al.*, 2001). To determine the N added to soil through sludge application, it is not only necessary to quantify the total N applied but also the N release rate, which can be done with an incubation trial.

The aim of this study is to quantify the N-mineralisation and N nitrification rates when different levels of sewage sludge are applied to a soil, and to compare it to similar levels of inorganic N from commercial fertilizer.

3.2. MATERIALS AND METHODS

The incubation trial was done in a laboratory, under constant temperature and moisture conditions. Soil (50 g) was placed in plastic jars and pre-incubated before the different treatments were applied.

3.2.1. Materials

i. Treatments

Treatments consisted of a set of different quantities of sewage sludge and commercial fertilizer applications (Table 3.1). Each treatment was replicated three times. The sludge was applied at the equivalent of 5, 10 and 20 ton ha^{-1} dried sewage sludge per 20cm cultivation depth. The commercial fertilizer (Limestone ammonia nitrate, LAN, 28% N) treatments were calculated based on the assumption that only 30% of the total N content of the sludge would be available in the short term (WRC, 1997).

Table 3.1: Different treatments and corresponding N concentrations

Treatment	**Total N**	**Available N** [2]
20 ton ha^{-1} sludge	628kg N ha^{-1}	188.4 kg N ha^{-1}
20 equivalent fertilizer	188.4 kg N ha^{-1}	188.4 kg N ha^{-1}
10 ton ha^{-1} sludge	314 kg N ha^{-1}	94.2 kg N ha^{-1}
10 equivalent fertilizer	94.2 kg N ha^{-1}	94.2 kg N ha^{-1}
5 ton ha^{-1} sludge	157 kg N ha^{-1}	47 kg N ha^{-1}
5 equivalent fertilizer	47 kg N ha^{-1}	47 kg N ha^{-1}
Control	0	0

ii. Soil

A dark red sandy clay loam topsoil (0-30 cm) from the Hatfield Experimental farm, University of Pretoria (25° 45'S 28° 16' E) from the same site that was later used for a field trial, was used. The soil was dried overnight at 40°C and passed through a 2

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[2] Availability of N is based on values from the 'Permissible utilisation of sewage sludge' (1997), that states only 30% of total N in sludge is available in the first year.

mm sieve. Some of the chemical and physical properties of the soils are presented in Table 3.2:

Table 3.2: Some physical and chemical properties of the soil used for the incubation trial

pH (H_2O)	6.4
Electric conductivity (mS m^{-1})	8.3
NO_3^--N (mg kg^{-1})	3.55
NH_4^+-N (mg kg^{-1})	1.37
C %	0.63
% Clay	26
% Silt	8.2
% Sand	65.8

iii. Sewage sludge

The sludge that was used for the incubation trial was obtained from the Olifantsfontein, WWTP of ERWAT. The sludge was air dried, ground and sieved through a 2 mm sieve.

Generally sludge from Olifantsfontein WWTP has low concentrations of heavy metals, because it is from a domestic origin and contains very little industrial waste. Some of the chemical properties of the specific sludge are presented in Table 3.3:

Table 3.3: C and N quantities of sludge

Total N	3.14%
Total C	3.63%
C:N	1.15

According to Hansen & Djurhuus, (1978), during mineralisation, N-release takes place when the C:N ratio is less than approximately 25, whereas immobilisation of N takes place at a C:N ration greater than approximately 35. It can therefore be expected that due to the relatively low C:N ratio of the sludge used, low N-immobilisation would occur.

3.2.2. Methods

i. Field capacity of the soil:

According to the literature, incubation studies are best done when the soil is at field capacity. To determine the field capacity (FC) of the soil, three open-end glass cylinders (40 cm × 3.5 cm) were used. One end of the cylinder was covered with filter paper, and filled with 5cm of soil. This layer of soil was compacted, until it resembled the density of the natural soil. Another 5 cm soil layer was added and compacted. This process was repeated until the cylinder was two thirds full. Water (25 ml) was added to each cylinder and a rubber stopper was placed loosely on top, to prevent evaporation. The cylinders were left for 24 hours, until the wetting front stopped moving. Samples were taken from the middle of each cylinder, weighed and dried at 100°C for 24 hours, and weighed again. The mean value of the water content (15.29%) was assumed to be FC.

ii. Pre-incubation trial

A pre-incubation step was carried out to ensure that the microbial population adapt to the experimental conditions. The soil was incubated at FC for one week as follows: plastic jars (300 ml) were filled with 50 g of the prepared soil and 7.65 ml of water was added to each jar, to bring the soil to FC. The samples were placed in the incubator, at a constant temperature of 21°C. Every second day the jars were opened for the soil to aerate, and deionised water was added to maintain FC. This was done for 7 days and on day 7, triplicate samples were taken to be analysed for NH_4^+ and NO_3^-, which were used as the background information for the trial, and were assigned 'Day 0'.

iii. Incubation

After the 7 days pre-incubation, on 'Day 0', dried sewage sludge and commercial fertilizer were applied according to the different treatment levels, mixed well, brought to FC and put into the incubation oven. The same procedure of aeration and water addition, which was carried out during the pre-incubation period, was repeated during the incubation trial.

Triplicate samples were taken at day 1, 3, 7, 14, 28, 42 and 63, and were analysed for NH_4^+ and NO_3^-.

iv. *NH_4^+ and NO_3^- analysis:*

Soil analyses were carried out according to Black *et al.*, (1965). 100 ml of a 1 M KCl solution was added to the 50 g soil samples and shaken for an hour, and filtered. From this filtrate, a 50 ml aliquot was used for the analysis of NH_4^+ and NO_3^-. To this aliquot, 20 ml of a 50% NaOH solution was added to volatilise the NH_4^+-N. The sample was distilled in a Büchi distillation unit and the steam volatilised was bubbled through a boric acid solution, with a colour indicator. After distillation, the boric solution was titrated with HCl. The volume of the HCl used for the titration was used to calculate the NH_4^+-N content in the incubated soil. To determine the NO_3^--N content, Devarda's alloy was added, to reduce the NO_3^- to NH_4^+. The sample was distilled again to determine the NH_4^+ that originated form the NO_3^--N

v. *Statistical analysis*

Statistical analyses were carried out on the results by using GLM program as a random blockless design. Comparisons between the different treatments, the intervals, as well as treatment and interval interaction were tested. Significant differences between treatments, and grouping were tested by the Tukey test.

3.3. RESULTS AND DISCUSSION

In practice it is not advisable to use a single value for the N content of sludge. During the drying process prior to sludge analysis, N losses may occur due to volatilisation and leaching (Sommers, 1977). Each sludge source and even batches therefore have to be considered individually because the N and therefore the total N supply differ.

3.3.1. Ammonium (NH_4^+)

Most heterotrophic soil microorganisms are able to produce NH_4^+ (Mengel & Kirkby, 2001; Coyne, 1999) therefore the first N-product of mineralisation will be NH_4^+. Additions of organic material, rich in readily available energy, stimulate mineralisation of organic N and C. The stimulating effect of an added source of energy on the microorganism activity and the subsequent increase in organic matter mineralisation is called 'priming effect' (Jansson & Persson, 1982; Kuzyakov *et al.*, 2000). The priming effect may start either immediately or shortly after the addition of organic matter to the soil. This will usually depend on the quantity and the availability of organic N in the sludge that can be used by microorganisms as well as the complexity of the organic N molecules in the sludge (Kuzyakov *et al.*, 2000). The priming effect can be observed in the production rate of NH_4^+ during this incubation trial (Figure 3.1).

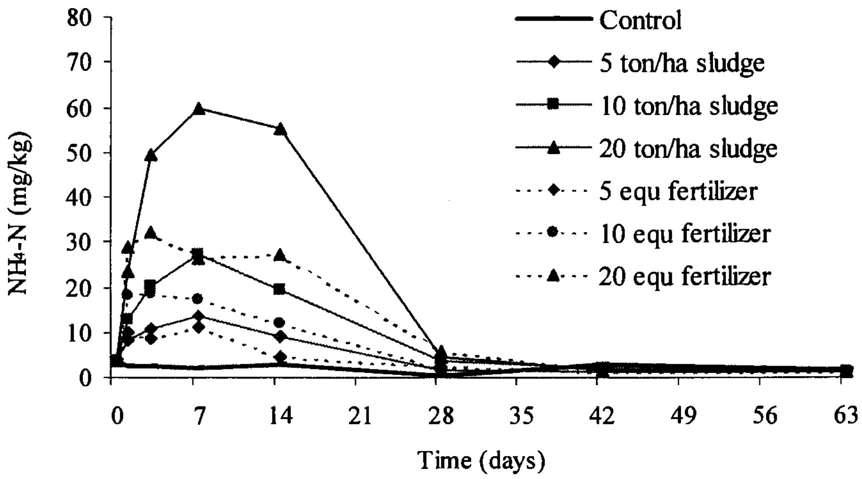


Figure 3.1: Total extractable NH_4^+-N content as a function of incubation time and different sludge and fertilizer N application

Samples were taken on day 0, before the different treatments were applied, and again on day 1, 24 hours after application. The sharp increase in NH_4^+-N content between day 0 and 1 in the sludge treatments can partly be ascribed to the free NH_4^+-N content in the sludge and fertilizer.

In soils, microbial activity is mostly limited by the energy source. According to studies done by Bosatta & Agren (1995), addition of inorganic fertilizer to the soil resulted in no change in microbial activity. Application of sludge though, greatly stimulates microbial activity (Jansson & Persson, 1982). The N applied in the inorganic fertilizer treatments amounts to 30% of the total N content compared to the corresponding sludge treatment. An increase in microbial activity because of the larger organic-N pool and readily available energy (C-source) from the sludge treatments can be possible reasons for the greater NH_4^+-N production from the sludge than from the fertilizer treatments. The total NH_4^+ concentration continue to increase, until it peaks at day 7, after which the concentrations decrease again. After 28 days, most of the NH_4^+ is depleted in the treatments.

The release of NH_4^+ only happens after the NH_4^+ requirements of the microorganisms are satisfied. The decomposition of microbial biomass eventually also contributes to the releases of NH_4^+ (Mengel & Kirkby, 2001; Coyne, 1999).

The presence of NH_4^+-N in the treated soil with applied NH_4^+ or by mineralisation of organic matter can lead to different pathways in the N cycle:

i. It can be utilised for microbial growth,
ii. NH_3 volatilisation can occur
iii. NH_4^+ can be utilised as an energy source by autotrophs in the nitrification process (Paul & Clark, 1989).

According to Figure 3.1 the quantity of extractable NH_4^+-N in the sludge and inorganic N applied treatments differ significantly ($P<0.0001$) from the control treatment. For all the treatments, except the control, a clear increase in NH_4^+ (priming effect) can be observed, immediately after the addition of the different treatments. The differences between the sludge and inorganic N application can be attributed to the different free NH_4^+ applied in the treatments and its effect on the microbial activity. There was a significant difference between all the treatments, except between the 5 ton ha^{-1} sludge and the corresponding 5 ton ha^{-1} fertilizer N treatment. At this low concentration, no significant differences were obtained, probably due to the small differences in the applied inorganic N in the sludge and commercial N applications.

3.3.2. Nitrate (NO_3^-)

The priming effect in production of NO_3^--N was small in comparison to the priming effect on the production of NH_4^+-N. The same observations were also made by Kuzyakov *et al.*, (2000). There were initially small quantities of NO_3^--N in the sludge treated soils while the NO_3^--N in the fertilizer treatments were high due to the inorganic applied NO_3^--N (Figure 3.2). The delay in NO_3^--N production, especially in the sludge treatments, represents the time necessary for the microbial population to increase to an extent sufficient to cause an increase in the nitrification rate, as also found by Alexander (1961). The two-step nitrification process can be expressed by the following reactions (Singer & Munns 1992):

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^-$$

Overall reaction: $$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad (1)$$

Depending on the redox conditions in the soil, the oxidation process of NH_4^+, can also be described as follows (Schmidt, 1982):

$$\begin{tikzcd} NH_4^+ \arrow[r, "2e"] & NH_2OH \arrow[r, "2e"] & {[NOH]} \arrow[r, "2e"] \arrow[d] & NO_2^- \arrow[dl] \\ & & N_2O & \end{tikzcd} \qquad (2)$$

In the oxidation of $NH_2(OH)$ to NO_2, an intermediate compound, [NOH] can form, that can be converted to either NO_2 under aerobic conditions, or to N_2O under more anaerobic conditions (Schmidt, 1982). The loss of N through N_2O-gas during the nitrification process is unaccounted for in this incubation trial, and can probable explain the difference in the applied N and the measured N.

According to Figure 3.2, the NO_3^--N content increases slowly in the early stage of incubation, while NH_4^+-N is produced and increased rapidly after 7-14 days at the expense of the NH_4^+-N content.

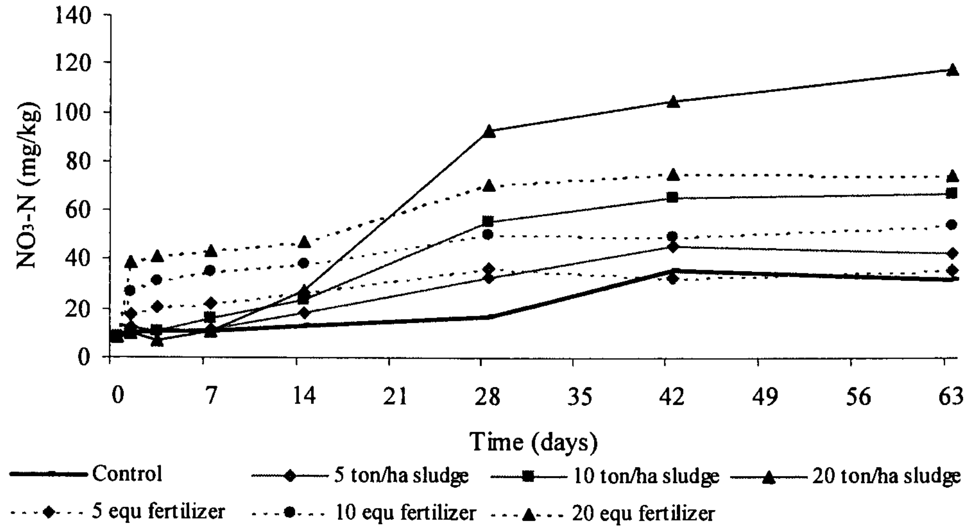


Figure 3.2: Total extractable NO_3^--N content as influenced by differential N application and incubation time

In the sludge treatments, no initial increase in NO_3^--N production was measured. After 7 days NO_3^--N levels increased until day 28, when production stabilised. This corresponds well with the NH_4^+ production, and it is evident that NO_3^--N is dependent on the NH_4^+ production. Similar results were obtained by Tester *et al.* (1977), where the mineralisation rate of organic material was measured. A decrease in NH_4^+ was observed, with a subsequent increase in NO_3^- levels.

In the fertilizer treatments, most of the NO_3^--N was immediately available (day 1). This is due to the inorganic nature of the commercial fertilizer. The increases in NO_3^--N level after day 1 could be due to the nitrification of NH_4^+ that was also present in the fertilizer.

3.3.3. Daily NO_3^--N production from sewage sludge

The NO_3^--N production during the different incubation periods for the different treatments is presented in Figure 3.3 to 3.5. These values were obtained by calculating the difference between the extractable NO_3^--N contents between different incubation periods, using the data from Figure 3.2.

According to Figure 3.3 to 3.5, the trend in NO_3^--N production for the different N applications levels was the same. The NO_3^--N content on day 1 can be assumed to be the NO_3^--N that was either supplied through the sludge and fertilizer applications or the natural NO_3^--N content of the soil itself.

At the 5 ton ha^{-1} application rate, Figure 3.3a, an instant increase in the fertilizer treatment can be observed, 24 hours after application. The extractable NO_3^--N in the sludge treatment increased slowly, and peaked at day 28.

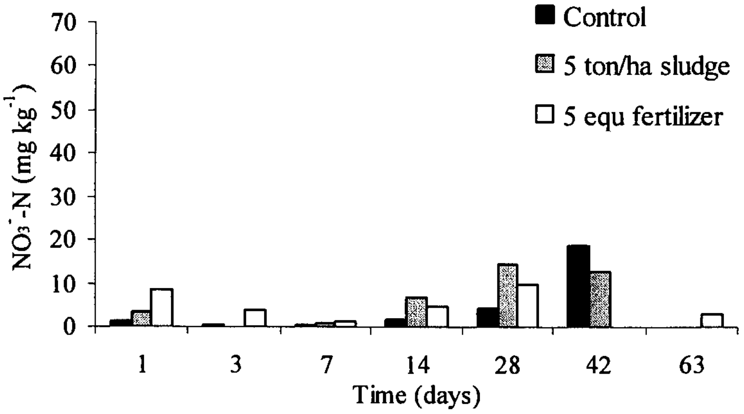


Figure 3.3a: NO_3^--N production between sampling periods for the 5 ton ha^{-1} sludge and fertilizer treatment

A daily production of NO_3^--N were also calculated, by dividing the NO_3^--N content by the number of days prior to previous sampling, and this is presented in Figure 3.3b:

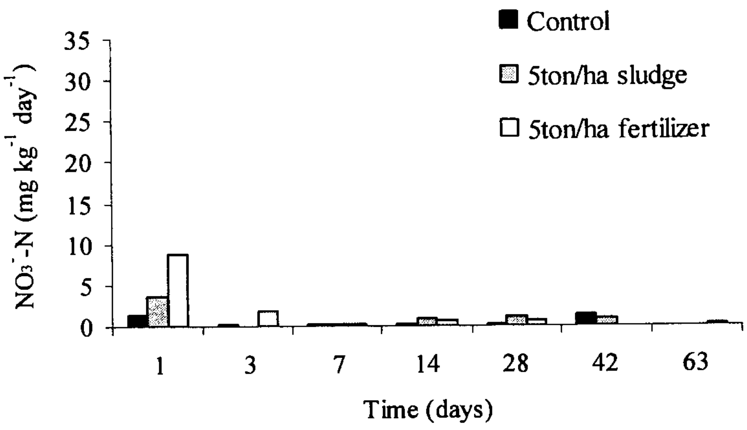


Figure 3.3b: Daily NO_3^--N production at 5 ton ha^{-1} sludge and fertilizer treatments

Here again the daily production of NO_3^--N after day 1 was low and increased slowly for all the treatments. The daily NO_3^--N production peak between day 28 and 42 and then decreases again. This lag of NO_3^--N production coincided with the decrease in NH_4^+-N content due to the microbial oxidation of NH_4^+. A sudden increase in inorganic N from the fertilizer treatment is visible after only 24 hours, with little or no additional NO_3^--N production after day 3.

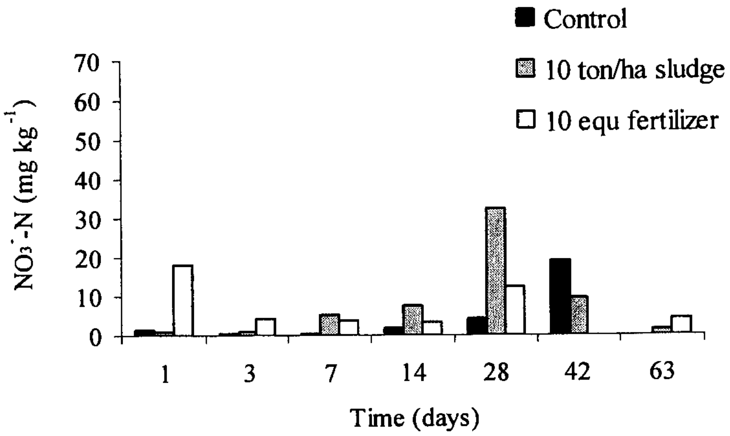


Figure 3.4a: NO_3^--N production between sampling periods for the 10 ton ha^{-1} sludge and fertilizer treatment

The daily production of NO_3^--N can be expressed as follows:

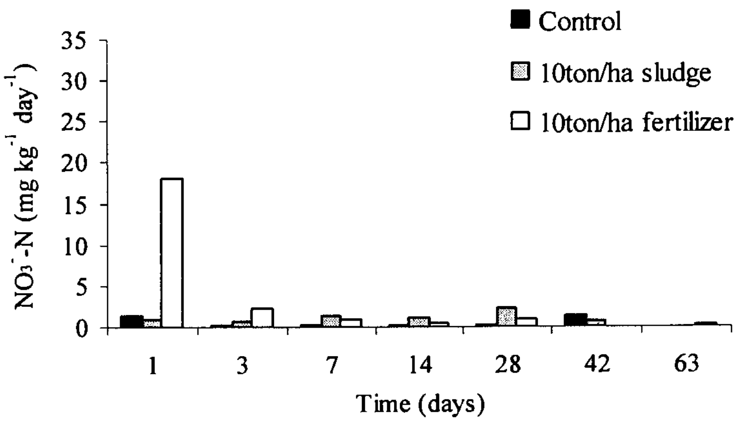


Figure 3.4b: Daily NO_3^--N production at 10 ton ha^{-1} sludge and fertilizer treatments

At the 10 ton ha^{-1} fertilizer and sludge treatment, the same tendencies in NO_3^- content and daily production rate were observed. The peaks, content and production rate were lager due to more N applied through the sludge and fertilizer.

At the 20ton ha^{-1} application rate (Figure 3.5a and 3.5b), this effect is most enhanced.

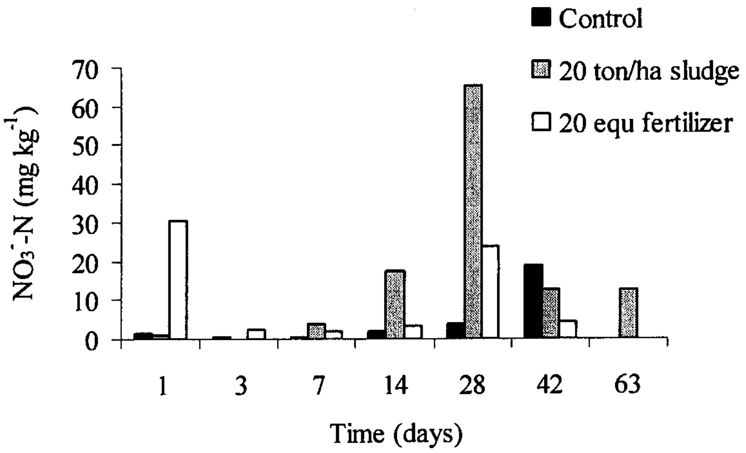


Figure 3.5a: NO_3^--N production between sampling periods for the 20 ton ha^{-1} sludge and fertilizer treatment

And the daily NO_3^--N production rate:

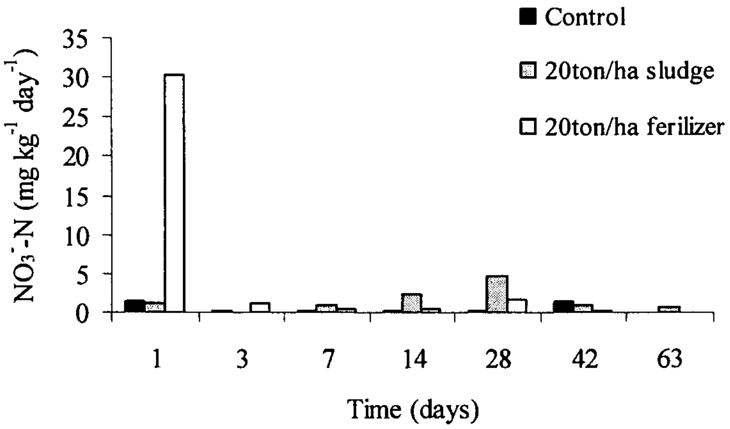


Figure 3.5b: Daily NO_3^--N production of 20 ton ha^{-1} sludge and fertilizer treatments

Here it can be seen, that directly after application of fertilizer and sludge treatments, more NO_3^- from commercial fertilizer is present compared to the sludge treatment. Hereafter, the NO_3^- production available is low, but steadily increases until day 28, and decreases again.

The sewage sludge follows a different trend. Initially the concentrations of NO_3^- from sludge is extremely low, and close to zero. This is because most of the N is still present in the organic form, that can be mineralised by microorganisms, before the N can become available as also found by Singer & Munns, (1992) and Korentajer, (1991). Here again the decreases in NO_3^- after 28 days indicate that the oxidation rate of NH_4^+ has slowed down and depleted. The high fraction of available NO_3^- from the commercial fertilizer on day 1 indicates the potential hazard of NO_3^- leaching from fertilizer.

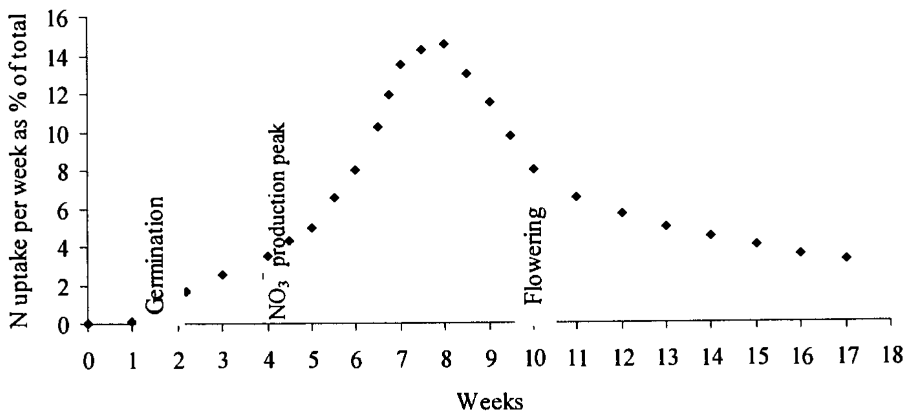
Table 3.4: Total extractable NO_3^--N and daily production during 63 day incubation period from 20 ton ha^{-1} sludge and its equivalent fertilizer treatment

Days	**0**	**1**	**3**	**7**	**14**	**28**	**42**	**63**	**Total**
				Sludge, 20 ton ha^{-1}					
Cumulative NO_3 production (mg)*	8.46	9.63	6.68	10.42	27.70	92.73	105.34	117.82	
Differences between interval (mg)*	8.46	1.18	-2.95	3.73	17.29	65.02	12.61	12.49	117.8
NO_3 production per day (mg)*		1.18	0.00	0.93	2.47	4.64	0.90	0.59	
				Fertilizer at 20ton ha^{-1} equivalence					
Cumulative NO_3 production (mg)*	8.46	38.81	41.31	43.40	46.89	70.57	75.13	74.59	
Differences between interval (mg)*	8.46	30.35	2.50	2.09	3.49	23.68	4.56	-0.54	74.6
NO_3 production per day (mg)*		30.35	1.25	0.52	0.50	1.69	0.33	0.00	

* All values are based on 1 kg mass base

From the daily NO_3^- production the total quantity of NO_3^- from sludge that becomes available over 63 days in the 20 ton ha^{-1} treatment was calculated (Table 3.4). This added up to 117.82 mg NO_3^--N kg^{-1} and 74.59 mg NO_3^--N kg^{-1} for sewage sludge and commercial fertilizer respectively. Although the total quantity of NO_3^- production in the fertilizer treatment is much less, the fractions of which these become available are not equally distributed, as one can see from Figure 3.5 a and b. After day 7 the rate at which NO_3^- becomes available from sewage sludge exceeds the rate of NO_3^- production from the fertilizer, but the *total* NO_3^--N released from sludge only exceeds the NO_3^--N release from the fertilizer treatment at day 28. This can indicate that more than the expected 30% of N in sludge are mineralised during this period (this will be quantified in Chapter 6).

The advantage sludge has over fertilizer, is that most of the NO_3^- only becomes available between day 14 – 28 of incubation. In practice it can be expected that NO_3^--N production can be lower than the incubation values, because in the incubation trial conditions are always optimal for the microorganisms. This fraction of N can therefore be utilised much more efficient by plants, that will reduce the risk of leaching. It is still not the ideal situation, because the maximum N utilisation by maize is at week 7 to 8 (49 – 56 days), just before flowering (Figure 3.6) (MVSA, 1997). However, when it is compared to commercial fertilizer, the N from sewage sludge is available at a more convenient time for the plant compared to the commercial fertilizer.



Adapted from MVSA, 1997

Figure 3.6: Rate of N uptake by maize during the growth period

3.3.4. Total inorganic release

Changes in inorganic N reflect the net mineralisation of organic N (Bernal *et al.*, 1998). By measuring the total release of NH_4^+ and NO_3^- over time, the mineralisation and nitrification rates of the specific sludge can be calculated. By subtracting the N release by the control treatment for every treatment, only the inorganic N release was obtained without background values. The total N release from the treatment that received 20 ton ha^{-1} sludge are presented in Figure. 3.7.

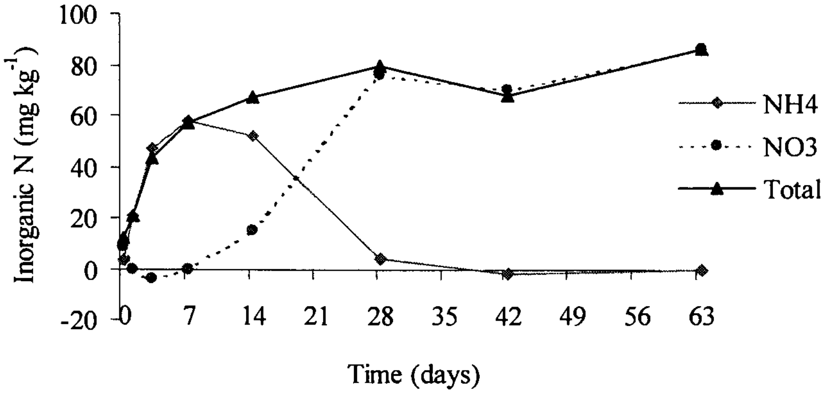


Figure 3.7: Changes in the NH_4^+-N, NO_3^--N and total N content during incubation of the 20 ton ha^{-1} sludge treatment

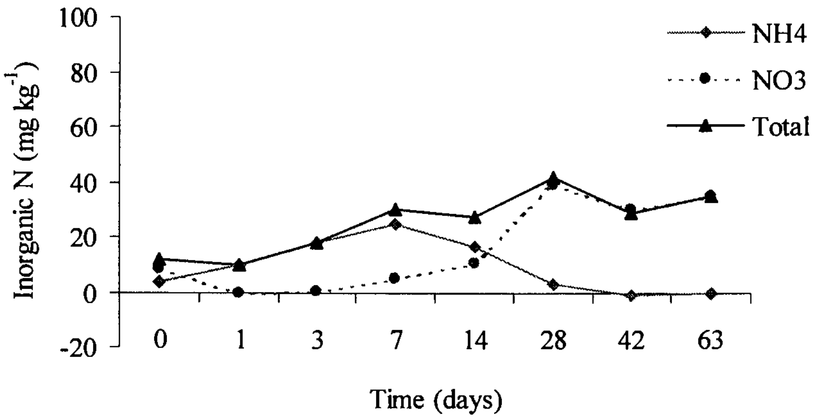


Figure 3.8: Changes in the NH_4^+-N, NO_3^--N and total N content during incubation of the 10 ton ha^{-1} sludge treatment

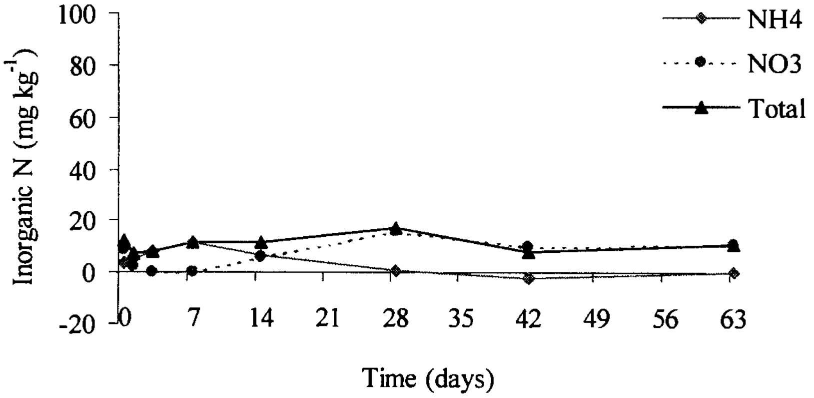


Figure 3.9: Changes in the NH$_4^+$-N, NO$_3^-$-N and total N content during incubation of the 5 ton ha^{-1} sludge treatment

According to Figure 3.7, at the 20 ton ha^{-1} sludge treatment the total N-content increases rapidly for the first 3 days, and N release becomes more steady after that. For this treatment, the 20 ton ha^{-1} represented 0.33 g of dried sludge per 50 g of soil as explained previously. This calculation is based on the assumption that the sludge is incorporated 20 cm deep in the soil. The total organic-N content of the sludge used, was 3.14%. This brings the initial N added to 10.36 mg per 50 g of soil, or 207.24 mg kg^{-1}. At the end of the incubation period (63 days), the total inorganic N produced was 117.82 mg kg^{-1}. The control treatment was subtracted (31.81 mg kg^{-1}) to give only the sludge mineralisation value of 86.01 mg kg^{-1}. This is an indication that 41.5% of the total organic N in the sludge was mineralised after 63 days.

At the 10 ton ha^{-1} sludge treatment (Figure 3.8) the total inorganic N after 63 days was 34.89 mg kg^{-1} (after subtracting of control values). The initial N added in the form of sludge, is 103.76 mg kg^{-1}, which indicates that 33.63% of the organic N was mineralised. This is in the same region as the 30% mineralisation in the first year, suggested by the 'Permissible utilisation and disposal of sewage sludge' (WRC, 1997).

At the 5 ton ha^{-1} treatment (Figure 3.9), an inorganic N release of 21.5% was obtained. The reason for the decrease in inorganic N release with the lower application and higher values with the high application rates can probably be ascribed to the extent the microorganisms were stimulated. High application rates add a larger extractable energy source to the soil, which microorganisms can exploit. This increases the microbial activity and the sludge can be decomposed at an accelerated rate.

The rate of decomposition of organic matter in sludge is highly variable, depending on the sludge composition (Korentajer, 1991). Different values of mineralisation rates have been obtained in the past. Mineralisation rates of organic N found in aerobically digested sewage sludge have been found to range from 20-50 % (Cripps *et al.*, 1992) and 4-48% during a 16-week incubation trial by Parker & Sommers (1983) and Magdoff & Chromec (1977).

It would therefore be incorrect to generalise the inorganic N concentrations of sludges due to the preparation processes, the sampling techniques and handling prior to analysis (Parker & Sommers, 1983). Soil type, sewage sludge source and climatic factors can also have significant effects on organic N mineralisation rates (Cripps *et al.*, 1992; Parker & Sommers, 1983; Korentajer, 1991).

3.4. CONCLUSION

The data obtained in these experiments showed that more N (mg kg^{-1} soil) is available from sewage sludge over time, than from commercial fertilizer. The rates at which the inorganic N becomes available from sludge and fertilizer is not equally distributed. Most of the N in sludge is organically bound, and not immediately available. N only becomes available after some time, through mineralisation. In the case of commercial fertilizer, which is an inorganic fertilizer, most of the N is immediately available.

From an agricultural point of view, the slow release of N holds numerous advantages. While the efficiency of commercial N fertilization is limited by factors such as high

NO_3^- leaching losses, or NH_4^+ volatilisation, the use of sludge as a slow release N material may decrease these N losses and increase N availability.

In the case of commercial fertilizer, the largest fraction of N is immediately available. The largest fraction of NO_3^- from sludge only becomes available between 14-28 days. This fraction of NO_3^- can be utilised much more efficiently by crops, which need more N during this period than at the germination period. Large fractions of NO_3^- in the soil at any given time are prone to leaching. The later the NO_3^- becomes available and overlap with the crop N needs, the better the N efficiency, and the lower the risk of NO_3^- leaching.

The sludge mineralisation rates obtained during 63 days ranged from 21.5% (5 ton ha^{-1}), 33.63% (10 ton ha^{-1}) to 41.5% (20 ton ha^{-1}). No specific values can be made, due to the influences of a number of factors, such as soil type, climatic factors, and sludge type. At this stage accurate N balances cannot be made, due to unmeasured N losses such as denitrification and NH_4^+ volatilisation.

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CHAPTER 4

NO_3^- leaching under field conditions

4.1. INTRODUCTION

Disposal of sewage sludge tends to be an increasing problem, due to scarcity of landfill sites and environmental concerns. On the other hand, another world-wide problem is the gradual decrease in the organic matter content in cultivated soils. In warm areas (like South Africa) organic matter decomposes faster than colder areas (Cripps *et al.*, 1992; Metzger & Yaron, 1987; Garcia-Gil *et al.*, 2000). This is a concern, due to the subsequent decrease in soil quality and stability. Constant applications of adequate quantities of organic matter are important to maintain or improve the productivity of soils that become low in organic matter content due to cultivation.

The question is: can the two problems not solve each other? The application of sewage sludge on soils with decreasing organic matter could be an economic and effective practice to improve soil quality and to dispose of sludge at the same time. By incorporating sewage sludge, the microbial activity of these soils can also be increased. Microbial activity and soil fertility are generally closely related because microorganisms release inorganic plant nutrients through mineralisation (Garcia-Gil *et al.*, 2000; Korentajer, 1991). In South Africa, with its warm climate and low soil organic matter, the addition of sludge to soil can have many advantages.

Scarcity of landfill sites, high-energy requirement for incineration and increasing costs of commercial fertilisers led to the consideration of sewage sludge application to agricultural lands as a practical alternative for waste disposal (Hue *et al.*, 1988). This practice is regarded as an inexpensive and environmentally acceptable way of sludge disposal (Terry *et al.*, 1979). The sustainability of such practices however, depends on sludge properties and loading rates as well as the chemical and mineralogical characteristics of the soils to which the sludge is applied (Hue *et al.*, 1988).

The potential hazards associated with sewage sludge include accumulation of heavy metals, pathogens, toxic organic components, and NO_3^- leaching (Korentajer, 1991). The NO_3^- content is often considered to be the limiting factor when disposing sludge

(Brye *et al.*, 2001). NO_3^- poses pollution risks due to NO_3^- leaching to groundwater or gaseous losses to the atmosphere (Mitchell *et al.*, 2000). In this chapter, the problem of possible NO_3^- leaching is addressed, to ensure sustainable application of sewage sludge to agricultural land, without the negative environmental impact of groundwater contamination. Strict guidelines currently exists on the disposal of sewage sludge that is based on the potential NO_3^- leaching hazard. However, no limitations are applicable to the use of inorganic N-fertiliser that can result in the same negative effects. Application rates limitations of sludge also did not take into account the effect of crops on NO_3^- leaching.

The aim of this study was to compare the potential NO_3^- production and leaching between sludge and commercial N fertiliser that is applied to soils under field conditions in the presence and absence of crops.

4.2. MATERIAL AND METHODS

To study the potential NO_3^- production and leaching due to applied sewage sludge, two field trials were done: one during the winter and one during the summer months.

i. *Location and climate*

This field trial was carried out on the Hatfield Experimental farm, of the University of Pretoria (25° 45'S 28° 16' E). This region receives summer rainfall, and the climate is classified as moderate and suitable for crop production. The average climatic data of the past 40 years is given in Table 4.1.

Table 4.1: Climatic data for the Hatfield experimental farm of the University of Pretoria

	Jan	**Feb**	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
Rainfall (mm)	131.7	85.7	83.7	53.9	13.4	7.3	2.5	4.9	19.1	20.5	105.9	122.1
Min Temp	16.1	15.9	14.5	11.1	7.0	3.7	3.7	6.3	10.5	12.9	14.2	15.3
Max Temp	28.1	27.9	26.6	24.2	21.8	19.1	19.4	22.0	25.6	26.6	26.7	27.5
Ave Temp	22.1	21.9	20.6	17.7	14.4	11.4	11.6	14.2	18.1	19.8	20.5	21.4

Source: Institute for Soil Climate and Water, Pretoria

ii. Soil

The soil at the experimental site is classified as a Hutton form according to the SA classification system (Departement van Landbou Ontwikkeling, 1991) and can be described as a red, apedal, sandy clay loam, the same soil used for the incubation trial. Some physical and chemical properties of the soil are given in Table 3.2, Chapter 3.

iii. Sewage sludge

Two different types of sludge were used for the winter and summer trial respectively. This was simply for practical reasons, and the availability of the sludge at the time of the trial. This did not influence the trial, because the N content in each sludge was taken into account separately for each trial. For the winter trial, sludge from the Benoni WWTP was used. Chemical composition of the sludge is given in Table 4.2.

Table 4.2: Chemical composition of Benoni sludge, expressed on a dry mass base

pH	6.36
Total N	2.22%
Total C	4.8%
C:N ratio	2.16
Total P	2.57%
% Water	55%

For the summer trial, sludge from the Olifantsfontein WWTP was used. Some chemical properties of the sludge are summarised in Table 4.3.

Table 4.3: Chemical composition of Olifantsfontein sludge (dry mass base)

pH	6.8
Total N	2.36%
Total C	6.43%
C:N ratio	2.72
Total P	0.21%
% Moisture	10.55

The main difference between the two sludges is that the sludge from the Benoni WWTP has a higher heavy metals content, due to the industrial origin, whereas Olifantsfontein WWTP sludge is from domestic origin which has low heavy metal content. The difference in moisture content was also taken into account and compensated for in calculations for application levels. The other properties are fairly similar.

iv. Experimental layout and treatments

A plot of 181 m × 18.5 m was subdivided into 12 blocks of 18.5 m x 12 m, with a buffer zone of 3 m between the blocks to prevent contamination. The winter and summer trials were done on the same area but on different blocks.

The winter trial started during May 2001 and was monitored until end of July 2001. This trial consists of six treatments as presented in Table 4.4.

Table 4.4: Treatments and levels of application for the winter trial

Number	**Treatment**	**Equivalent (per hectare)**	**Total N per ha**
1	Control	0	0
2	Fertiliser	35kg/ha LAN	10 kg N/ha
3	Sewage sludge	4 ton/ha	88.8 kg N/ha
4	Sewage sludge	8 ton/ha	177.6 kg N/ha
5	Sludge + crop	8 ton/ha	177.6 kg N/ha
6	Sewage sludge	16 ton/ha	355.2 kg N/ha

The experimental layout was a randomised block design with two replicates as shown below.

5	1	4	6	2	3	2	6	3	1	5	4
Replicate 1						Replicate 2					

Of the six treatments, only four could be used. The sludge + crops treatment was not effective and was discarded due to crop failure. The fertiliser treatment was discarded because it was situated on a shallow, stony patch that made sampling impossible. Therefore only different levels of sludge treatments were compared under field conditions.

The summer trial was done on the same area, starting middle October 2001 and continuing until middle January 2002. The blocks were smaller and their placements were carefully selected to avoid areas where there were constrains in the soil as indicated below:

	1	3	*(part left out because of shallow soil)*	1		3
	2	4		2		4

These block sizes were 12 m x 7 m with the 3 m between the blocks. Four treatments were applied and replicated two times as given in Table 4.5.

Table 4.5: Different treatments and levels of application for the summer trial

Number	**Treatment**	**Equivalent (per hectare)**	**Total N per ha**
1	Control	0	0
2	LAN Fertiliser	1.68 ton/ha	470 kg N/ha
3	Sewage sludge	20 ton/ha	470 kg N/ha
4	Sludge + crop	20 ton/ha	470 kg N/ha

For this trial, the total N application, except for the control, was kept constant but higher than before to simulate a worse case scenario. The sludge was equally spread on the soil surface by hand, and disced approximately 10 cm into the soil.

v. *Sampling procedures*

Soil samples were taken every 2 weeks, at depths of 0-30 cm, 30-60 cm and 60-90 cm, with a soil auger. Two samples were taken on each plot, and the samples from the same depths were thoroughly mixed. For the different sampling times, samples were taken on different sites in the blocks. Sampling continued for three months, because it was expected that total mineralisation of the applied sludge would have taken place during this time.

To determine the possible NO_3^- leaching, the soils samples were taken up to 90 cm, and compared over time. Special attention was given to the 30-60 and 60-90 cm depth, because it was assumed that the change in NO_3^--N content at these depths could be an indication of NO_3^- leaching. Large fluctuations were expected in the 0-30 cm depth, due to the atmospheric influences in the topsoil, mineralisation, nitrification and denitrification. In the presence of a crop, the influence of NH^+_4-N and NO_3^- uptake would also cause variation.

vi. *Laboratory analysis*

After the samples were collected, the moisture content was determined gravimetrically by oven drying the samples overnight at 40°C. The soil was sieved through a 2 mm sieve. 24 hours after sampling, 50 g samples of the soil were extracted with 100 ml 1 M KCl. After being shaken for an hour, the samples were filtered through a 2V Whatman filter paper. A 50 ml sample of the filtrate used for the analysis of NH_4^+ and NO_3^- by standard laboratory methods, using a Büchi distillatory as discussed in Chapter 3.

vii. *Statistical analysis*

Analysis of variance (ANOVA) of the data was done on the statistical program SAS. The Tukey test was used to determine the least significant differences between treatments ($\alpha = 0.05$).

4.3. RESULTS AND DISCUSSION

The results are discussed in terms of the NH^+_4-N and NO_3^--N content, which were measured at different times and depths. According to the literature, changes in the NH_4^+ and NO_3^- contents can be expected as detailed in i and ii below.

i. *NH_4^+-N*

It is expected that NH_4^+ would mainly be present in the upper horizons, and would decrease with soil depth. In the sludge treatments, the organic material would stimulate microbial activity, and the consequent mineralisation of organic N to inorganic NH_4^+-N (Mengel & Kirkby, 2001). Once NH_4^+ has been formed, there are a number of possible reactions that can take place:

a) it may be taken up by plants;
b) it may be immobilised by microorganisms;
c) NH_3 volatilisation can occur in alkaline soils;
d) it may be utilised as an energy source by a special group of autotrophs;
e) it may be adsorbed onto the exchange complex and later be replaced by cations in the soil solution;
f) it may be fixed between the interlayer of certain 2:1 clays; and/or
g) NH_4^+ could react with soil organic matter to form certain quinone-NH_2 complexes (Paul & Clark, 1989).

ii. *NO_3^--N*

At the early stages after the application of treatment the NO_3^- content is expected to be fairly low, but should steadily increase. The lag represents the time necessary for the microbial population to increase and subsequently increase mineralisation of organic matter and the production of NH_4^+ (Alexander, 1961). The NO_3^- content will then increase due to the nitrification process. Once NO_3^- is formed in the soil, it is subjected to the following reactions:

a) it may undergo denitrification by microorganisms to gaseous oxides of nitrogen and to N_2;
b) it may be utilised by microorganisms and plants as an N source;
c) it may be leached from the upper profile to deeper soil layers;

d) it may he transported from the site by runoff; and/or

e) it may accumulate in the soil under certain circumstances if none of the above is applicable (Paul & Clark, 1989).

The rate of nitrification will depend on environmental conditions such as temperature, O_2 supply, moisture, pH, available mineral and the C:N ratio (Alexander, 1961).

4.3.1. Winter Trial

During the winter trial a number of changes in the NH_4^+ and NO_3^- contents were observed.

i. NH_4^+-N

Due to the summer rainfall area in which the trial was situated, the precipitation during this time span was low. Apart from the low temperatures, the availability of irrigation water was limited, and this resulted in low soil moisture content, and a subsequent decrease in microbial activity was suspected. The release of NH_4^+-N by microorganisms during mineralisation was therefore also low, as is evident in Figure 4.1. The NH_4^+-N release stayed low during the trial, and no priming effect could be observed, as seen in the incubation trial.

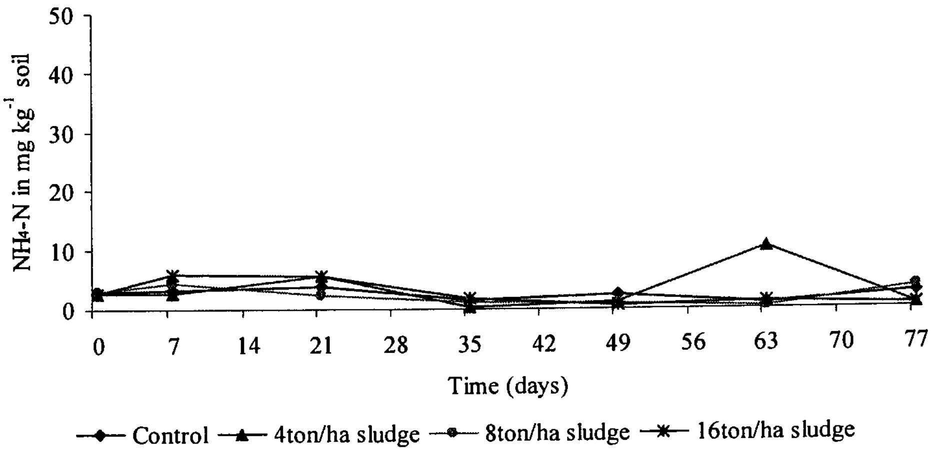

Figure 4.1: Changes in extractable NH_4^+-N content at 0-30 cm depths during the winter trial, as influenced by N-application and time

According to the statistical analysis, no significant differences were found between different treatments nor between different time intervals. There were also no significant differences between treatments at 0-30 cm (Figure 4.1), 30-60 cm (Figure 4.2) and the 60-90 cm (Figure 4.3) depths. The NH_4^+-N content in all the soil layers was very low in terms of NH_4^+-N content.

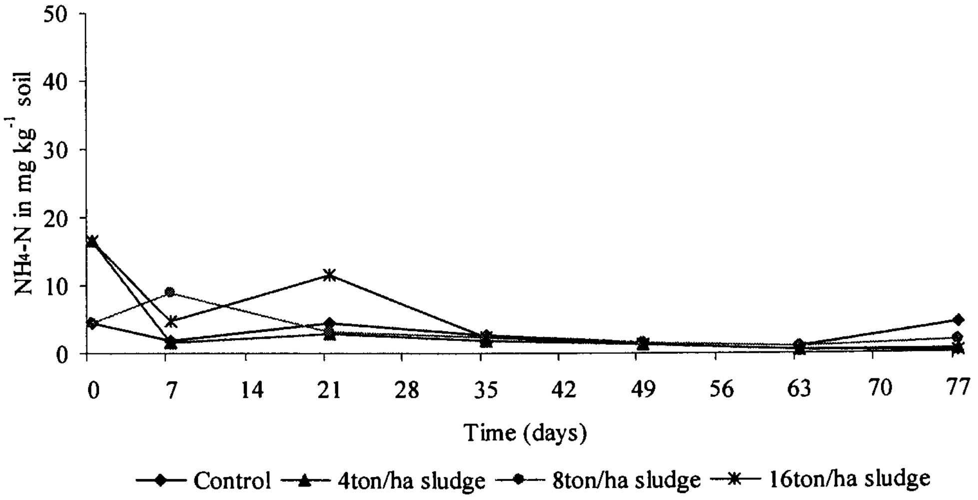

Figure 4.2: Changes in NH_4^+-N content at the 30-60 cm depth under winter circumstances as influenced by N-application and time

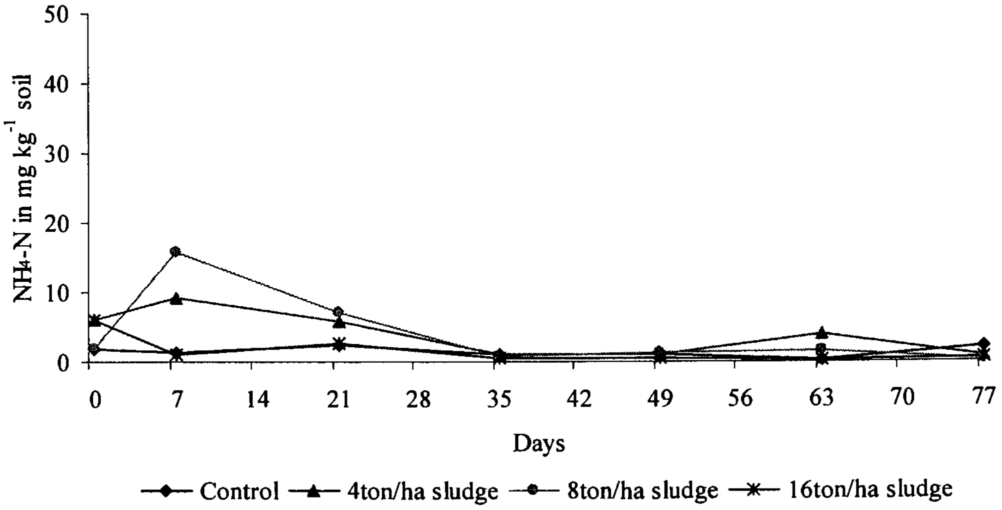

Figure 4.3: Changes in extractable NH_4^+-N content at 60-90 cm depth during the winter trial as influenced by N-application and time

As a result of the dry and cold climatic conditions, little or no mineralisation took place, which resulted in low NH_4^+ production. Subsequently the nitrification rate could also be expected to be low, and this will lower the possibility of available NO_3^--N for leaching.

ii. *NO_3^--N*

The NO_3^--N production in the soil is dependent on the NH_4^+-N content. For this trial, the NH_4^+-N production was limited due to the low moisture content and cool conditions in the soil during the winter months. The NO_3^--N production was therefore also limited, and relatively low (Figure 4.4) and did not differ significantly. After 50 days the NO_3^- content increased to a certain extend as a result of the irrigation.

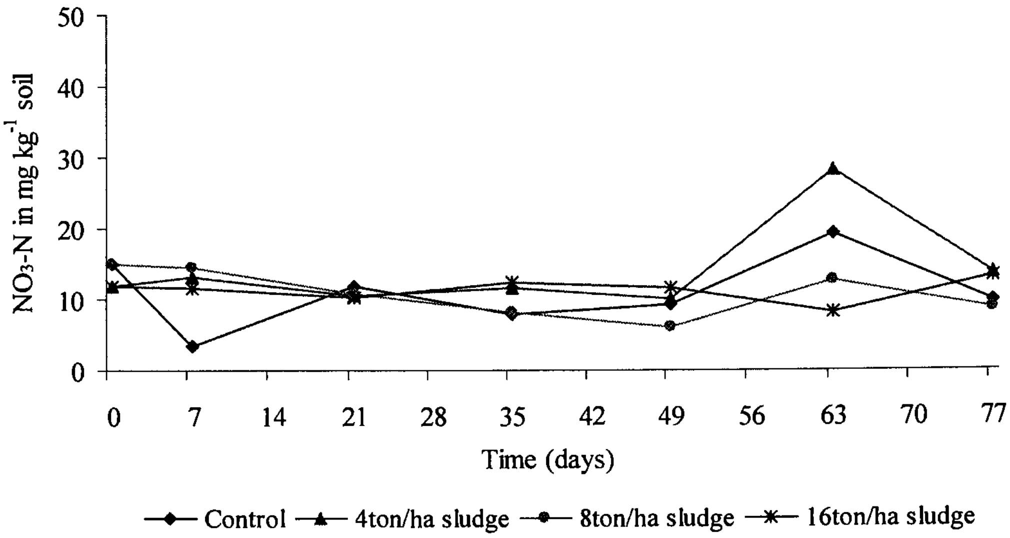

Figure 4.4: Changes in extractable NO_3^--N at the 0 – 30 cm depth for different treatments over time during the winter trial

According to Figure 4.5 there was an increase in NO_3^- content in the 30-60 cm depth at the higher sludge application rates, after 20 days. This indicates that some NO_3^- moved down in the profile and therefore disappeared from the 0-30 cm layer (Figure 4.4). This leached fraction appear in the 30-60 cm depth, but after 35 days this NO_3^- was probably redistributed in the rest of the profile, possible upwards due to upward capillary water movement because there was no evidence that it moved down to the 60-90 cm depth (Figure 4.6).

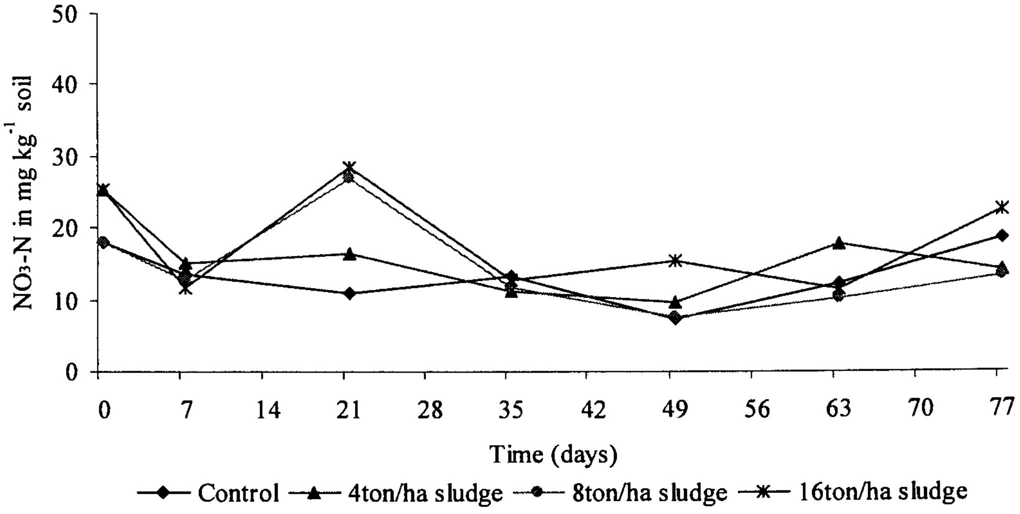

Figure 4.5: Changes in extractable NO_3^--N at the 30-60 cm depth for different treatments over time during the winter trial

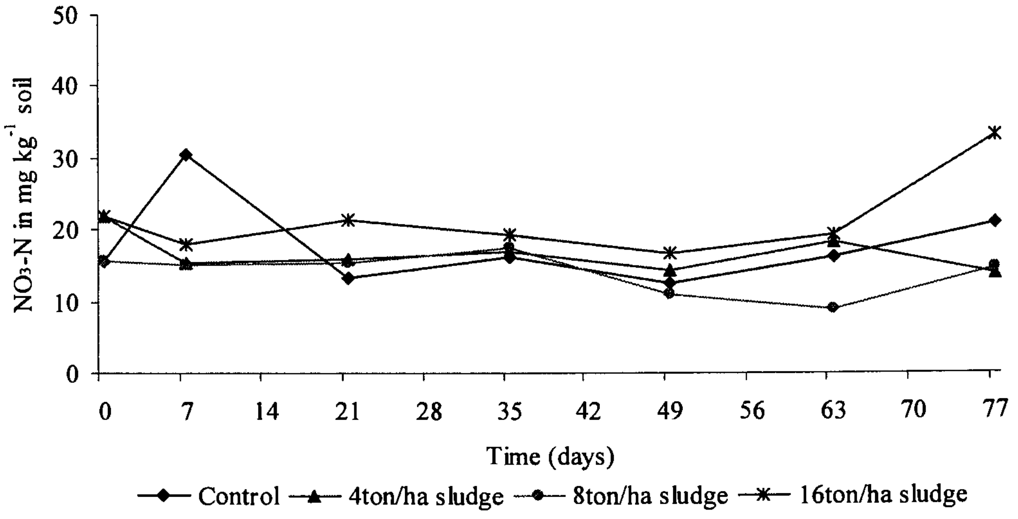

Figure 4.6: Changes in extractable NO_3^--N at the 60-90 cm depth for different treatments over time during the winter trial

4.3.2. Summer Trial

There were vast differences in the responses to the treatment during a similar trial done during the summer months. The maize crop that was used to simulate normal agricultural practices grew well.

i. *NH_4^+-N*

The results obtained from this experiment, were more distinct than that from the winter trial, due to the higher application rates that were applied, higher moisture content and higher soil temperatures. When necessary the plots were irrigated with an overhead irrigation system. The moist environment was more suitable for microorganisms, and the microbial activity increased correspondingly. The influences on the NH_4^+ production were much more prominent in the topsoil, 0-30 cm (Figure 4.7) compared to the subsoil, 30-60 cm and 60-90 cm (Figure 4.8 and Figure 4.9).

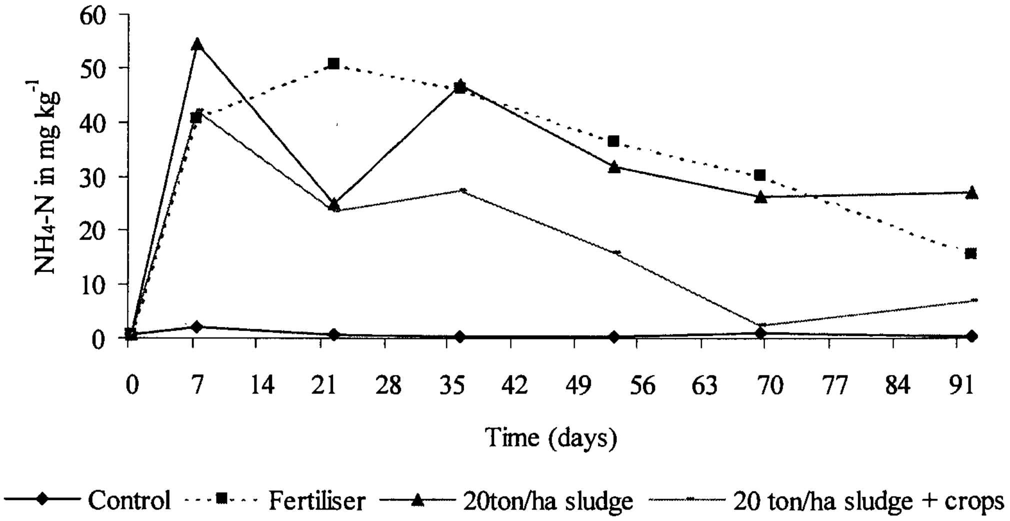

Figure 4.7: Changes in the NH_4^+-N content in the 0-30 cm soil layer due to the different treatments, under summer circumstances

Day 0 represents the natural NO_3^- content in the soil, before application of different treatment. The sludge and fertiliser were applied on day 0. During this time the maize was planted as well. After applying the fertilizer and sludge, there were dramatic increases in the NH_4^+ contents, as measured on day 7. This clearly illustrated the influence of the newly added sludge and fertiliser on the microbial activity. After day 7, the NH_4^+ content stayed constant for some time and then decreased. These decreases can be ascribed to nitrification of the NH_4^+-N. According to the statistical analysis, there are highly significant differences between the treatments ($P<0.0001$).

The 20 ton ha^{-1} sludge and equivalent fertiliser treatment responded similarly. Of importance is the lower NH_4^+-N content in the sludge plus crop treatment. At the early stages the crop was recently planted and its influence on the moisture content and NH_4^+-N uptake was minimal. As time passed the influence of the crop could be clearly observed because of the low NH_4^+-N content. This could be ascribed to the NH_4^+-N uptake of the plants or to lower microbial activity due to lower moisture content of the soil from evapotranspiration. Nitrification of NH_4^+-N can also contribute to the decreasing NH_4^+-N content in the soil. After 70 days the NH_4^+-N contents of the sludge + crop treatment were similar to the control, while there were still NH_4^+ present in the top soil in the fertilizer and sludge treatments. The steady decreases of NH_4^+-N in the treatments that was not planted could mainly be due to nitrification, while in the presence of a crop it could be due to both nitrification as well as NH_4^+-N uptake by the crop.

Microorganism activity decreases with depth due to a decrease in organic matter (Paul & Clark, 1989). NH_4^+-N content was also lower as a result of decreasing microbial activity, and this is illustrated in Figure 4.8 and 4.9. In the 30-60 cm layer, there were no significant changes in the NH_4^+-N content at the early stage of the trial. This clearly illustrates that there were no movement of NH_4^+-N to the deeper soil layers. This was in sharp contrast to the fertiliser treatment, that showed significant increases in the NH_4^+-N content after 35 days (Figure 4.8). N is leached mainly as NO_3^-, but in sandy soils, or where excessively high amount of NH_4^+-N is present, NH_4^+ can be also leached to some extent. This is a particularly serious problem in areas of shallow ground water table (Korentajer, 1991). In this case the excess NH_4^+ was probably leached down to some extent over time.

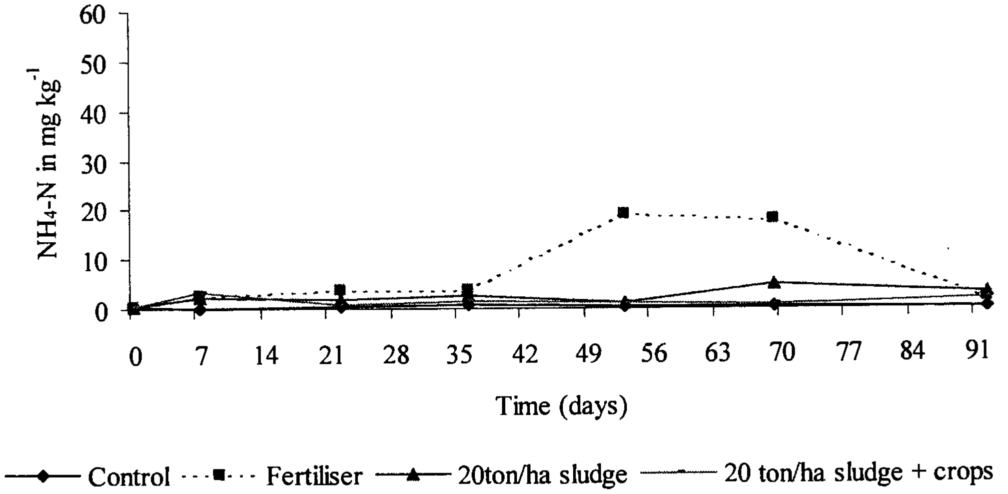

Figure 4.8: Changes in NH_4^+-N at 30-60 cm soil layer between different treatments, under summer circumstances

According to Figure 4.9, no NH_4^+-N was detected in the 60-90 cm soil layer. The insignificant increase in the fertiliser treatment on day 7 can be a result of contamination during the sampling or an experimental error.

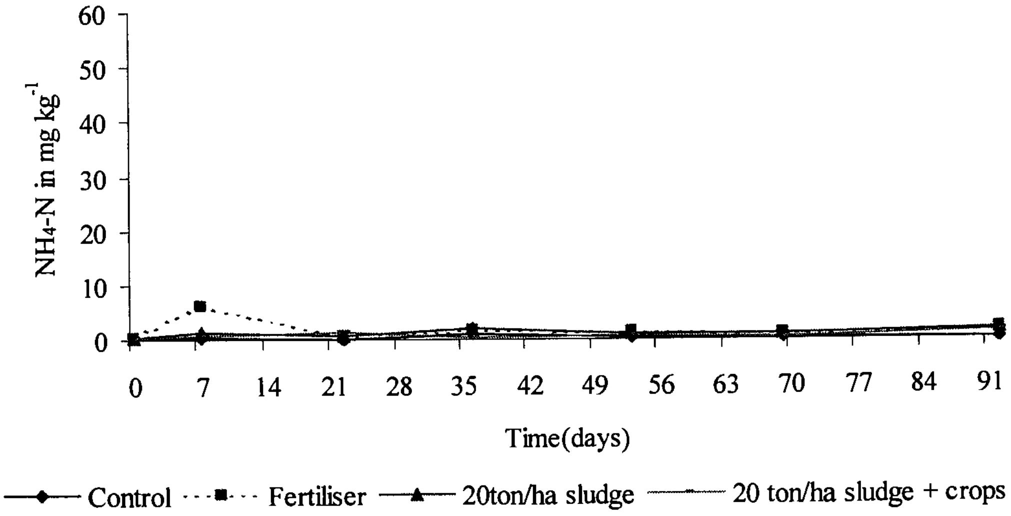

Figure 4.9: Changes in NH_4^+-N at 60-90 cm soil layer between different treatments, under summer circumstances

ii. *NO_3^--N*

Day 0 represents the background value of NO_3^--N, prior to treatment application. According to Figure 4.10, NO_3^--N applied in the form of LAN fertiliser, shows an immediate increase in NO_3^--N content after application and differs significantly from the other treatments.

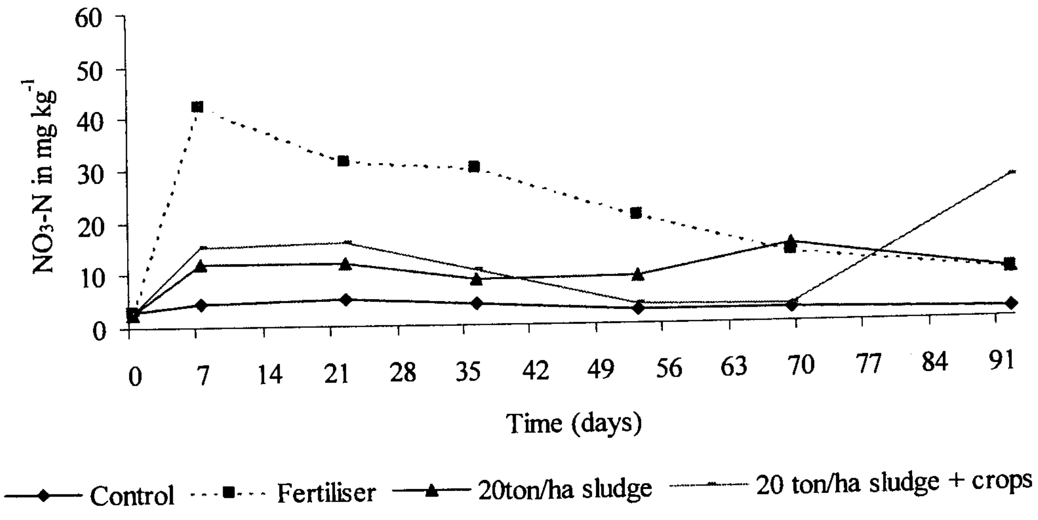

Figure 4.10: Changes in NO_3^--N at 0-30 cm between different treatments, under summer circumstances

Commercial fertilizer, that contains N already in the inorganic N form has a large portion of immediately available NO_3^--N. It steadily decreased after day 7 as it moved down the profile. The N applied in the form of sewage sludge only caused a slight increase in NO_3^--N content and kept constant at ± 10 mg kg^{-1}. N in sludge is mainly present in the organic form, which needs to be mineralised before it can be released in the inorganic form. It is possible that the rate of removal of NO_3^--N by percolating water is the same as the NO_3^--N production from NH_4^+-N by microorganisms. There were no significant differences between the sludge treatment and the sludge + crop treatment. The increase in NH_4^+ in the sludge + crop treatment after day 90, could be due to added organic material from the crops itself.

In Figure 4.11 shows that at the 30-60 cm depth, the content of NO_3^--N of the fertiliser treatment increased significantly over time up to day 55, after which it decreased again. The NO_3^--N fraction present in the 0-30 cm depth (figure 4.10), started leaching, and passed through the 30-60 cm soil layer (figure 4.11), resulting in the increase of NO_3^--N content. As it passed through this layer to the 60-90 cm soil layers (figure 4.12), the NO_3^--N content in the layer decreased again. The sludge treatments show no significant differences from the control.

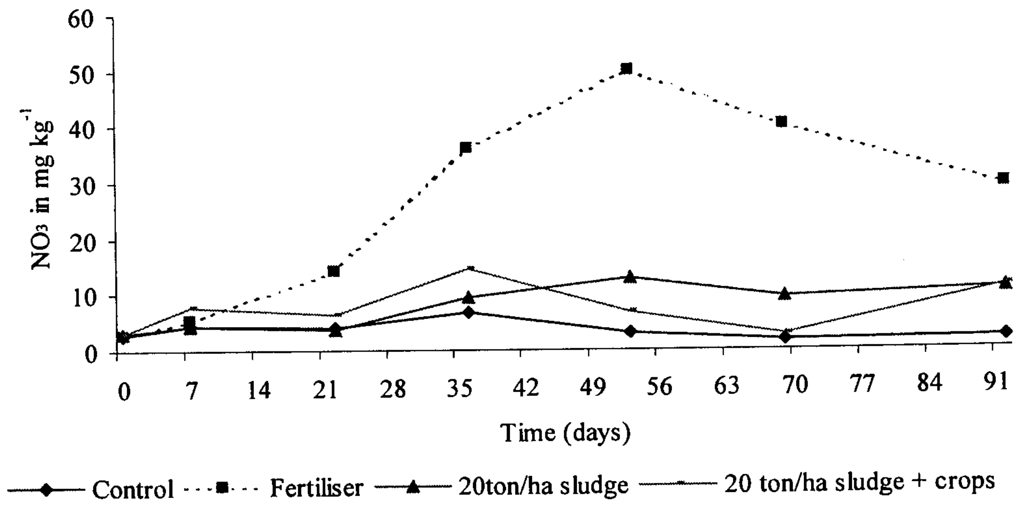

Figure 4.11: Changes in NO_3^--N at 30-60 cm between different treatments, under summer circumstances

According to Figure 4.12 the levels of NO_3^--N at the 60-90 cm were very low in all cases. Only the fertiliser treatment showed a slight increase after 53 days, but this increase only became significant at day 70. This increase corresponds with the decrease in NO_3^--N content in the 30-60 cm depth, where the NO_3^--N started to decrease at day 53, as the NO_3^--N fraction was removed through leaching to the 60-90 cm level.

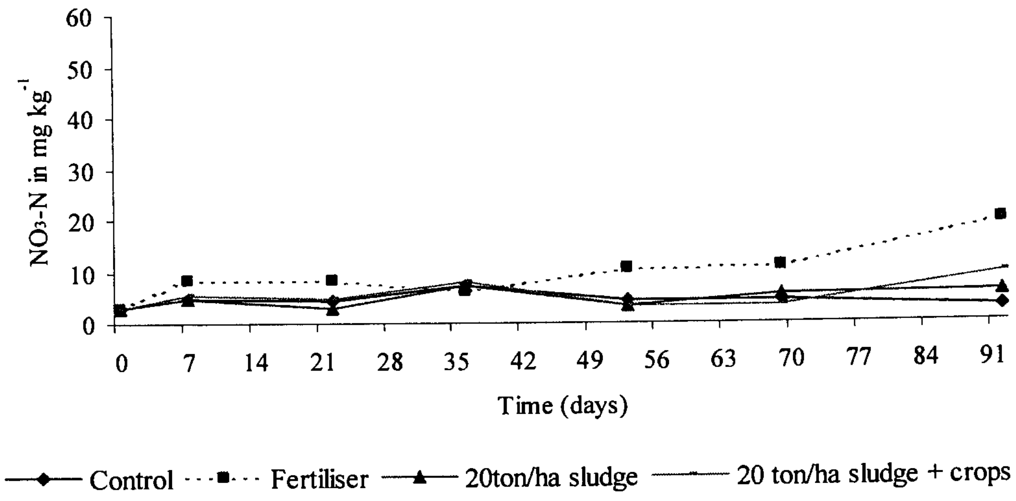

Figure 4.12: Changes in NO_3^--N at 60-90 cm between different treatments, under summer circumstances

4.3.3. NO_3^--N leaching and plant uptake

Accurate quantification of NO_3^--N leaching losses under field conditions is difficult. Movement of NO_3^--N within the soil depends on two factors: i) the concentration of NO_3^--N in the soil solution and ii) the water flux (Brye *et al.*, 2001). The problem of NO_3^- leaching is related to the use of N in crop production. During periods of low rainfall, evapotranspiration exceeds precipitation and no leaching is expected. If precipitation exceeds evapotranspiration, leaching may occur (Magdoff, 1992; Hansen & Djurhuus 1997).

Crop cover has a major influence on NO_3^- leaching and can be reduced if NO_3^- is taken up by a cash crop. Hansen & Djurhuus (1997) found that crops could make a significant difference to leaching. Different plant species have different uptake rates and patterns and would react to various N fertilisers in different ways (Kowalenko & Cameron, 1978). If sludge application rates are too low, insufficient N may result in low yields (Magdoff & Amadon, 1980); high application rates, on the other hand can lead to NO_3^--N leaching (Shepherd, 1996).

In the summer trial, the sludge treatment with and without crops can be compared. This can give an indication as to what extent the maize can reduce NO_3^--N from the sludge-applied soils. Figure. 4.13 shows the comparison in NO_3^--N content at different depths between the different treatments.

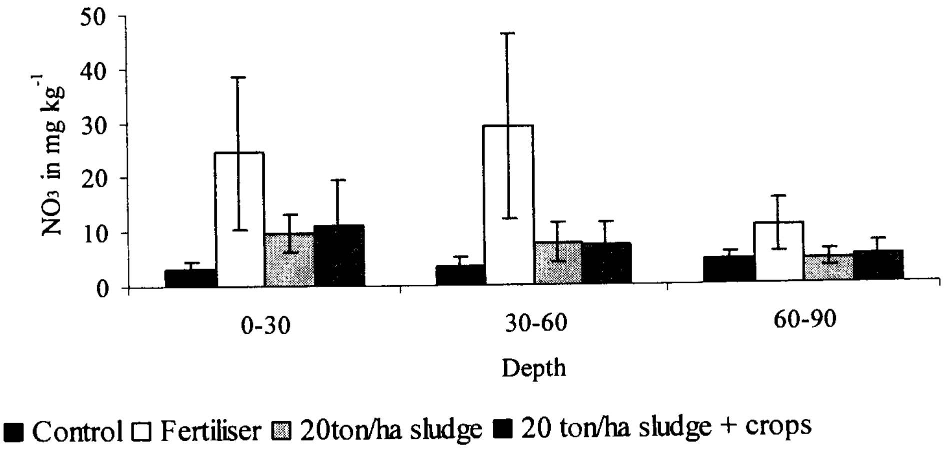

Figure 4.13: Average changes in NO_3^--N at different depths during 92 days of summer trial

The fertiliser treatment shows the highest NO_3^--N content throughout the profile, which is an indication of leaching. No significant differences were obtained between the average values of a) the sludge, b) sludge with crops and c) control treatment during the 92 days of the trial. The mean values of the sludge and sludge and crop treatment were more or less constant at all the depths. In Figure 4.14- 4.17 the different treatments are presented separately.

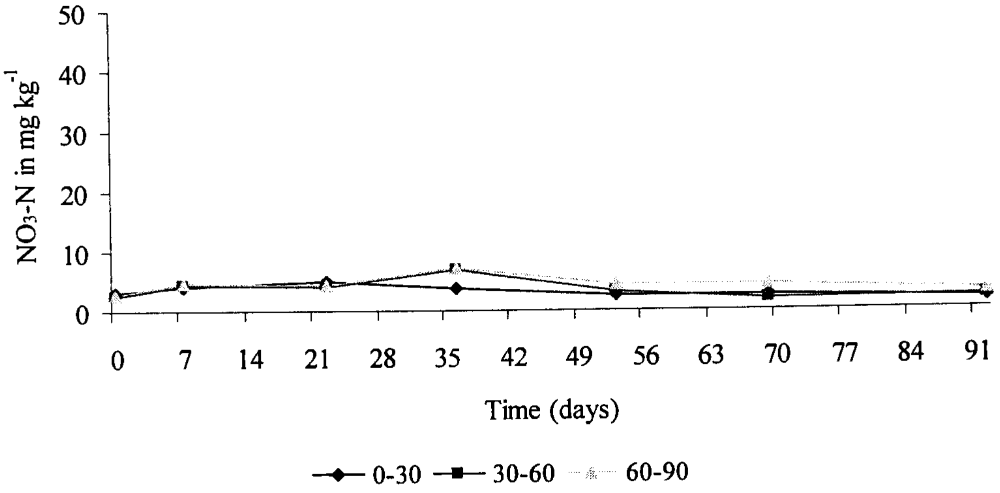

Figure 4.14: Changes in extractable NO$_3^-$-N at different depths in the control treatment, summer trial

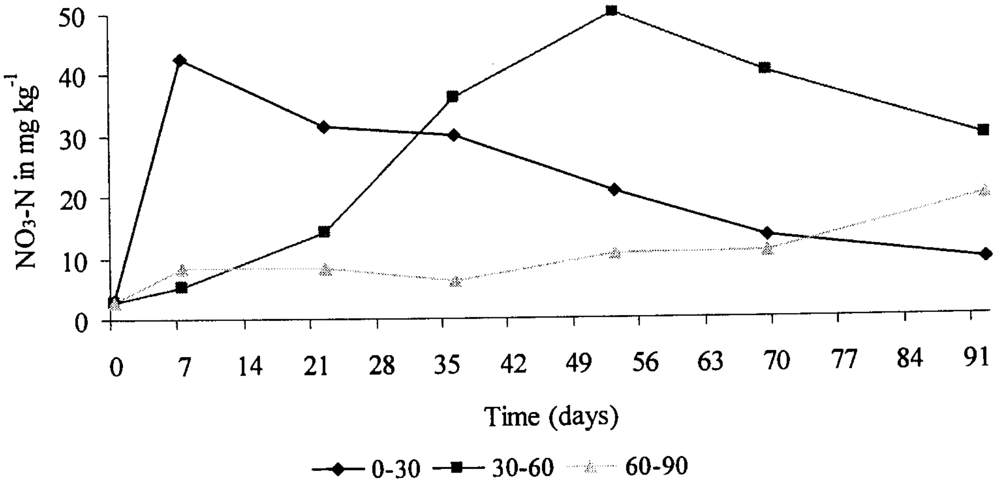

Figure 4.15: Changes in extractable NO$_3^-$-N at different depths in the fertiliser treatment, summer trial

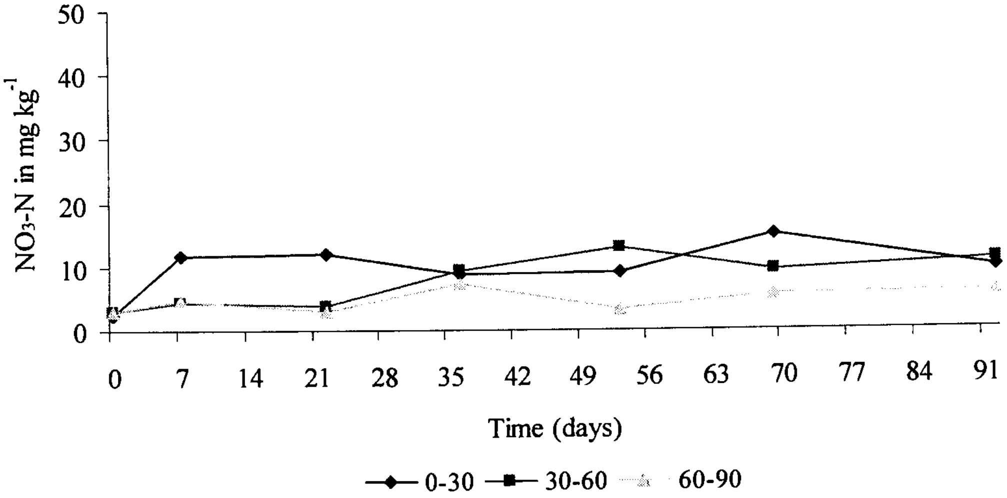

Figure 4.16: Changes in extractable NO_3^--N at different depths in the sludge only treatment, summer trial

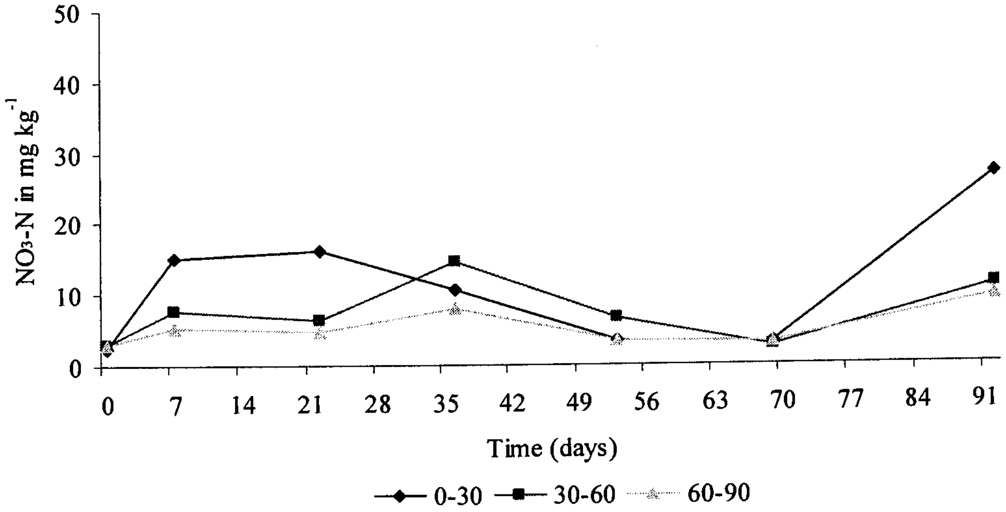

Figure 4.17: Changes in extractable NO_3^--N at different depths in the sludge and crop treatment, summer trial

The only graph that shows excessive quantities of leaching is the fertiliser treatment (Figure. 4.15). Shepherd (1996) obtained similar results, where NO_3^- leaching from commercial fertiliser was always higher than that from the sludge. The reason for this is that the N in fertiliser was fully available in the inorganic NO_3^--N form, whereas the N in the sludge became slowly available through mineralisation.

The sludge treatment without crops and the sludge treatment with crops showed no significant differences. It also did not differ significantly from the control treatment. The effect of vegetation on NO_3^--N leaching is therefore not evident in this experiment. This can be ascribed to the relatively low N release from sludge, and the fact that plants were still young at the beginning of the experiment. More distinct plant uptake differences will probably be observed at higher application rates. Similar results were reported by Shepherd (1996) who found that large losses would only occur when crop N needs has been exceeded. A decrease in leaching as a result of crops was also observed.

Mitchell *et al.* (2000) also found considerable variability in the soil inorganic N data, and ascribe these variations to consecutive sampling occasions that could vary markedly, and to N losses through unmeasured processes.

4.4. CONCLUSION

The following conclusions were made regarding NH_4^+-N and NO_3^--N production and leaching from sewage sludge and commercial fertiliser applied to the soil under field conditions:

- NH_4^+-N production from sludge is low in low moisture content, and low soil temperatures;
- Low NH_4^+-N levels result in low nitrification rates, and subsequently low NO_3^--N production;
- During the winter trial, little NO_3^--N leaching was observed, and the risk of NO_3^--N contamination to groundwater was low;
- During the summer months, with more soil moisture and higher soil temperatures, the NH_4^+-N production from sewage sludge as well as from fertiliser were initially

high possibly due to high microbial activities. This decreased over time mainly due to nitrification;

- Microbial activity is restricted to the upper horizons, and therefore also the NH_4^+-N content. NH_4^+-N decreases down the soil profile;
- NO_3^--N from fertiliser is initially very high, due to the inorganic form in which it is applied. NO_3^--N from sludge only becomes available over time, due to nitrification;
- Leaching of NO_3^--N under the fertiliser treatment occurred immediately due to the large concentration of inorganic NO_3^--N available;
- There are no significant differences between any of the sludge application loads and the control treatment; and,
- Sewage sludge applied at the same concentrations in terms of N content, did not show any significant leaching during the 92 day summer trial, whereas the fertiliser treatment showed significant leaching up to 60-90 cm depth.

The field trial results clearly illustrate the inadequacy of present guidelines, which stipulates strict N application levels for sludge, but none for commercial fertiliser. Even though the maximum loading rate for sludge is 8 dried ton ha^{-1} year^{-1}, no leaching occurred in 20 ton ha^{-1} sludge treatment. Commercial fertilizer on the other hand showed very high leaching levels up to 60-90 cm depth.

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CHAPTER 5

N_2O emissions from sludge and commercial fertilizer

5.1. INTRODUCTION

WWTP treats large volumes of domestic and industrial wastewater, and in the process produce sewage sludge as a by-product. Application of sludge to agricultural land has been proved to be an economical and effective way of disposing sludge. It also has the environmental benefit of nutrient recycling (Lerch *et al.*, 1990). There are, however, a number of restrictions. N in the sludge can become an environmental pollutant (Gaines & Gaines, 1994), either in the soluble form, NO_3^- that can contaminate ground water, or in a gaseous form, N_2O, that will be discussed in this chapter.

N_2O is a greenhouse gas with important impacts on the environment. Compared to pre-industrial times, the increase in global emissions has been estimated at 50% (De Groot *et al.*, 1994; IPCC, 1996). Although its absolute concentration is low compared to CO_2, it may significantly contribute to the greenhouse effect due to its high IR absorbing capacity and its long residence time in the atmosphere (Vermoesen *et al.*, 1996). Global emissions of N_2O are directly related to land use and agricultural practises. Between half and two-thirds of all anthropogenic N_2O emissions are thought to come from cultivated soils (Scott *et al.*, 2002). Not only does the loss of N through N_2O have important environmental problems, but N losses can also lead to higher expenses for farmers (Wrage *et al.*, 2001; Scott *et al.*, 2002; Luo *et al.*, 1998).

N management is therefore an important aspect of sludge application, and researchers have been trying to balance the equation of N inputs and outputs, but there seems to be an imbalance of N in agricultural systems that cannot be accounted for. This has been a topic of investigations for more than 50 years, and it is still not clear what the fate of N is (Wrage *et al.*, 2001). Nitrification and denitrification is part of the natural N cycle. In a previous study done by Luo *et al.* (1998), it was found that the NO_3^- leaching losses were lowered due to possible denitrification of NO_3^- in upper horizons. The formation of N_2O therefore reduced the remaining NO_3^- that could be available for leaching and denitrification may minimize NO_3^--N pollution to groundwater (Luo *et al.*, 1998; Castle *et al.*, 1998). In return the N_2O emissions can contribute global warming, a problem that also needs attention.

The aim of this experiment is to determine the rate of N_2O emission from sewage sludge and commercial fertiliser enriched soils. These values can be compared to ammonification and nitrification values obtained in a previous experiment under similar circumstances. With this data it is expected to obtain N-balance equation that can be used for maximum efficient utilisation of sludge on agricultural land, without environmental pollution.

5.1.1. Formation of N_2O

The net emission of N_2O from soil depends on the rate of N_2O formation, the rate of diffusion out of the soil and the consumption of N_2O during denitrification (Webster & Hopkins, 1996). However the understanding of N_2O is still incomplete and the estimates of the contributions from different processes vary widely (Webster & Hopkins, 1996). Both abiotic and biotic processes may be of importance to the production of N_2O in the soil. The abiotic production may occur through chemodenitrification, while nitrification and denitrification are the main biotic processes (De Groot *et al.*, 1994). A short summary of the most important processes that results in N_2O as a product are given:

i. Autotrophic nitrification

Nitrification is the oxidation of NH_4^+ or NH_3 to NO_3^- via NO_2^-. These reactions are carried out by two groups of microorganisms: the first part up to NO_2^- is conducted by the so-called NH3-oxidizers or primary nitrifiers, and the second step is carried out by NO_2^- oxidisers or secondary nitrifiers (Wrage *et al.*, 2001).

ii. Heterotrophic nitrification

Apart from autotrophic nitrifiers using nitrification as an energy source for fixing CO_2 heterotrophic nitrification is also possible. Heterothophic nitrifiers use organic C as a C and energy source (Wrage *et al.*, 2001).

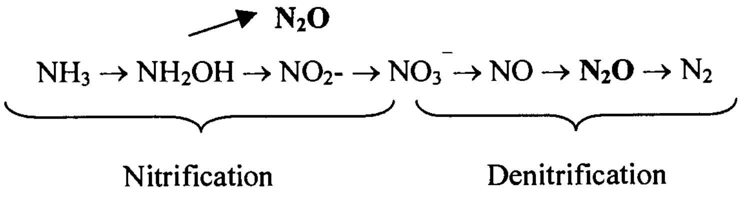
iii. Denitrification

The main origin of N_2O is the denitrification process. This can be defined as the process by which nitrogenous oxides, mainly NO_3^- and NO_2^- are used as terminal electron acceptors in the absence of O_2 and are reduced to dinitrogen gases during respiratory metabolism (Coyne, 1999). Denitrification is therefore the stepwise reduction of NO_3^- to N_2, with several intermediates (Wrage *et al.*, 2001).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (1)$$

iv. Nitrifier denitrification

A possible pathway of nitrification, called nitrifier denitrification might contribute to a large part of the loss of NH_4^+ from soil in the form of NO or N_2O. The oxidation of NH_3 to NO_2^- is followed by the reduction of NO_2^- to N_2O and N_2 (Wrage *et al.*, 2001). N_2O production by nitrifying bacteria may arise either from dissimilatory NO_2^- reduction when O_2 supply is limited, or during NH_4^+ oxidation to NO_2^- (Webster & Hopkins, 1996). This can be illustrated in as follows:



$$\underbrace{NH_3 \rightarrow \overset{\nearrow \mathbf{N_2O}}{NH_2OH} \rightarrow NO_2^- \rightarrow}_{\text{Nitrification}} \underbrace{NO_3^- \rightarrow NO \rightarrow \mathbf{N_2O} \rightarrow N_2}_{\text{Denitrification}} \qquad (2)$$

Nitrifier denitrification can be influenced by environmental, soil and biological factors such as pH, temperature, soil organic matter, microorganisms, rainfall, etc. (Scott *et al.*, 2002, Wrage *et al.*, 2001). The most important components are probably O_2 and NH_3 in the soil, and the number of active nitrifiers with the ability to denitrify (Wrage *et al.*, 2001).

In previous trials, the loss of N through denitrification was not measured, and could not be taken into account. This shortcoming was corrected in the denitrification trial where the rate of N_2O emissions from sewage sludge amended soils was determined. An experiment was done, measuring N_2O -N gas with a gas chromatograph. The aim of this study was to evaluate the possible N losses due to denitrification as influenced by sewage sludge and commercial fertilizer application, water content and time.

5.2. MATERIAL AND METHODS

i. Soil

A dark red sandy clay loam topsoil (0-30 cm) from the Hatfield Experimental farm, University of Pretoria (25° 45'S 28° 16' E) was used. The soil was dried overnight at 40°C and passed through a 2mm sieve. Some of the chemical and physical properties of the soils are presented in Table 5.1:

Table 5.1: Some physical and chemical properties of the soil used for the incubation trial

pH (H_2O)	5.1
NO_3^--N (mg kg^{-1})	2.9
NH_4^+-N (mg kg^{-1})	0.8
Textural classes	
% Clay	23.6
% Silt	1.5
% Sand	76.7

ii. Sewage sludge

The sludge that was used for the incubation trial was obtained from Olifantsfontein WWTP. The sludge was air dried, ground and sieved through a 2 mm sieve. Some of the chemical properties of the specific sludge are presented in Table 5.2:

Table 5.2: C and N quantities of sludge

Total N	2.815
Total C	1.205
C:N	0.428

iii. Treatments

Treatments consisted of a set of different quantities of sewage sludge and commercial fertiliser applications, as well as different moisture contents. Limestone ammonium nitrate (LAN) was used as commercial fertiliser. Each treatment was replicated three times. The sludge was applied at the equivalent of 10 and 20 ton ha^{-1} dried sewage sludge at 20 cm cultivation depth. The commercial fertiliser treatments were calculated based on the assumption that only 30% of the total N content of the sludge would be available in the short term (WRC, 1997).

To determine the effect of moisture content on the production of N_2O, two moisture levels, 50% and 100% field capacity (FC) were applied.

To differentiate between the between the different reaction of N_2O and N_2 production, three gas treatments were applied: 0kPa C_2H_2 (control) (A), 0.01kPa C_2H_2 (B) and 10 kPa C_2H_2 (C). Treatment A gives the net N_2O emission (total N_2O production –N_2 production), treatment B inhibits N_2O production during nitrification (prevent oxidation of NH_4^+ by autotrophic bacteria) and C inhibits N_2O formation during nitrification as well as denitrification (inhibits reduction of N_2O to N_2) (Webster & Hopkins, 1996). Values of N_2O-N emissions can be calculated accordingly.

(A-B)+C = total N_2O-N production;

((A-B)+C)-A = consumption of N_2O-N by denitrifiers;

(A-B) = total nitrification etc.

iv. Laboratory procedures

Flasks (200 ml) were filled with 25 g of soil. The soil was moisturised to specific moisture contents (50% and 100% FC), and pre-incubated for 7 days under a constant temperature of 21°C. The water content was kept constant during the experiment. After the 7 days pre-incubation, the sludge and fertilizer treatments were applied to the soil samples. 24 hours prior to measurement, the flasks were sealed off with rubber stoppers and the gas treatments were applied by injecting the C_2H_2 with a needle through the rubber stopper. Flasks were kept closed for the 24 hours until samples were analysed, but in between sampling, the flasks were left open to aerate. Measurements were taken on day 0, 1, 3, 7, 14, 21 and 28. To determine the N_2O content of the treatments, a 1 ml air sample was taken with a syringe from the headspace of soil samples and injected to the inlet of the gas chromatograph (GC). , and measured on a gas chromatograph. A Shimatzu gas chromatograph GC-14B fitted with an electron capture detector was used. The GC setting was 35°C for both the oven temperature and the column, and 300°C for the detector temperatures. Helium was used as a carrier gas with a flow rate of 55 ml min^{-1}. The column and detector temperatures were 35 and respectively. A standard N_2O source with a concentration of 24.5μL N_2O L^{-1} was used for instrument calibration and to evaluate the N_2O of the treatments.

v. Conversion of GC values to production rates

Different volumes of the standard gas were injected in the GC and the values obtained were used to draw the standard curve: GC values against a mass of N_2O. The slope of the standard curve was then used to calculate the N_2O values from all the treatments, which was expressed in μg kg^{-1} h^{-1}. Because the gas treatments were applied 24 hours prior to measurement, the values were divided by 24 to obtain a production rate per hour.

5.3. RESULTS AND DISCUSSION

A schematically representation of the pathway nitrifier denitrification is shown below in Figure 5.1.

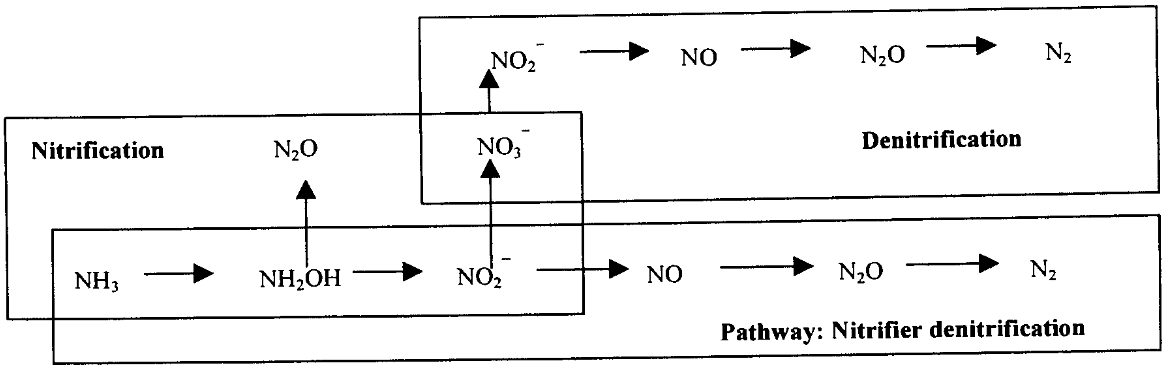


Figure 5.1: Nitrifier denitrification pathway (Adapted from Wrage *et al.*, 2001)

Low concentration of 0.01kPa C_2H_2 is known to inhibit oxidation of NH_4^+ and therefore inhibits N_2O production by autotrophic nitrifiers. Larger concentration of C_2H_2, (10kPa), inhibits the reduction of N_2O to N_2, therefore the consumption of N_2O by denitrifiers (Webster & Hopkins, 1996). Higher moisture content promotes denitrification, while dryer soil suppresses it. However, at 50 and 100% FC used in this experiment, no significant differences were obtained between the two moisture contents. For that reason, only the 100% FC treatments are discussed below.

The control treatment (A) gives an indication of the net N_2O emission rate (Figure. 5.2). This is an indication of greenhouse gas emission rates as a result of fertilizer and sludge application.

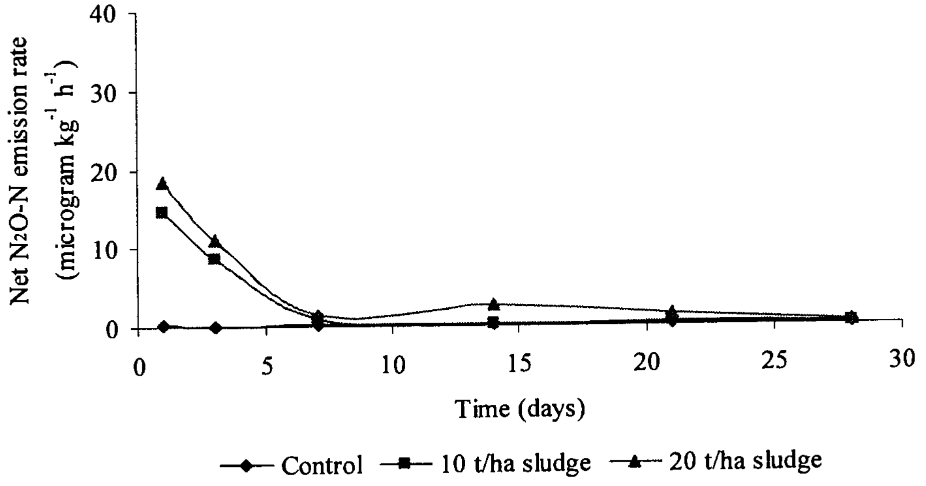


Figure 5.2: The net N_2O emission rate in µg kg^{-1} dry soil hour^{-1}

The net emission rate of N_2O -N decreased fast, and then stabilised. At the 10ton ha^{-1} treatment no N_2O -N emissions is measured after 7 days, while this only occur after 21 days in the 20ton ha^{-1} treatment.

A fraction of N losses through denitrification never reaches the atmosphere in the form of N_2O. In an anaerobic environment, N_2O can be even further reduced to N_2 by denitrifying organisms (Webster & Hopkins, 1996). This is directly dependant on the availability of N_2O -N, and correlates with the total production of N_2O -N in Figure 5.4. The rate at which these organisms reduced N_2O to N_2 is illustrated in Figure 5.3.

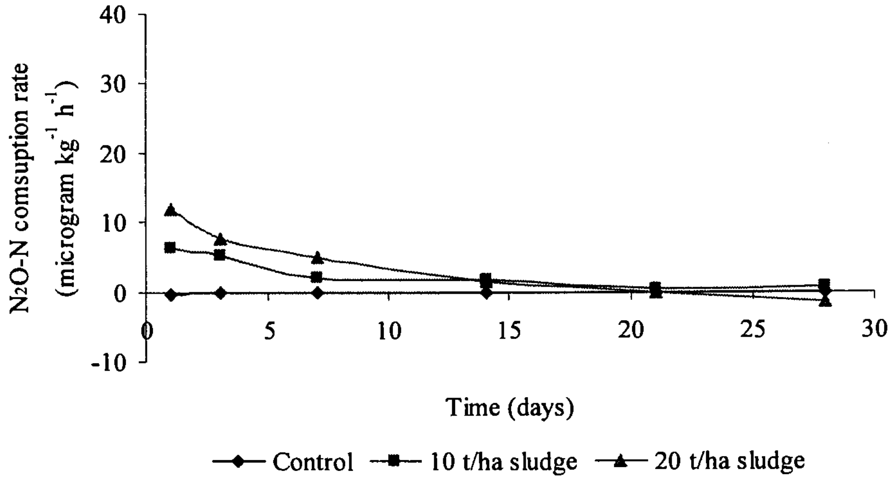


Figure 5.3: The rate of N_2O -N consumption by denitrifiers, in µg kg^{-1} dry soil hour^{-1}, over the extent of 28 days

N_2O that is lost through further reduction to N_2 by microorganisms (Figure 5.3) does not pose any hazard in terms of greenhouse emissions. Total losses of N in the soil due to denitrification, are the sum of the net production and consumption by microorganisms (Figure. 5.4).

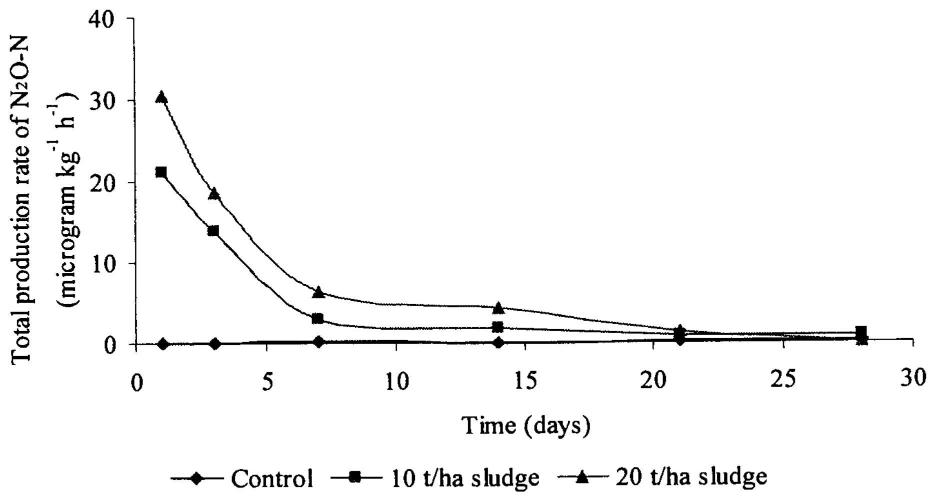


Figure 5.4: The total production rate of N_2O -N in µg kg^{-1} dry soil hour^{-1} over the extent of 28 days

The total production rate of N_2O-N initially decreased fast and then stabilised. The 20 ton ha^{-1} treatment produced 30 µg N_2O-N kg^{-1} dry soil h^{-1}. The production decreases over 21 days, until there are no significant N_2O-N emissions. The 10 ton ha^{-1} treatment followed the same trend, but started with a lower production of N_2O-N of 21 µg N_2O-N kg^{-1} dry soil h^{-1}. Very little N_2O emissions were detected after only 7 days. The production of N_2O is generally considered to be positively related to the availability of inorganic N (Vermoesen *et al.*, 1996). A larger concentration of mineralisable organic N in the higher application rates could therefore also influence the production of N_2O-N. After 21 days, no N_2O-N emissions were detected.

5.3.1. Denitrification values

The total production of N_2O obtained from the control, 10ton ha^{-1} sludge and 20 ton ha^{-1} sludge treatments at 100% FC is given in Table 5.3.

Table 5.3: Total production of N_2O in the 100%FC treatment from the control and sludge applications

Days	**1**	**3**	**7**	**14**	**21**	**28**
			Control			
Production rate (μg kg^{-1} h^{-1})	0.10	0.07	0.14	0.10	0.19	0.15
Daily production (g ha^{-1})	2.20	2.52	2.56	1.05	1.93	1.58
			10 ton ha sludge			
Production rate (μg kg^{-1} h^{-1})	21.04	13.94	2.969	1.81	0.92	0.84
Daily production (g ha^{-1})	1515.02	501.80	53.44	18.66	9.41	8.67
			20 ton ha sludge			
Production rate (μg kg^{-1} h^{-1})	30.342	18.64	6.381	4.205	1.252	0
Daily production (g ha^{-1})	2184.62	671.04	114.86	43.25	12.88	0

The total losses of N through denitrification can also be expressed in terms of N_2O losses per hectare. Table 5.3 shows that for the 10 ton ha^{-1} treatment, on day one, a total of 1.5 kg N_2O was lost per hectare. This decreased to a total daily loss of 0.5 kg ha^{-1} N_2O on day three. Similar trends occurred in the 20 ton ha^{-1} treatment, where 2.18 kg ha^{-1} N_2O was lost on day one, and 0.67 kg on day three. The N_2O losses seems small in comparison with losses from ammonification and nitrification (see Chapter 6).

5.3.2. Moisture influence on N_2O-N production

The water regime, which regulates the O_2 diffusion into the soil, is one of the main factors regulating the emission and consumption of N_2O (Bandibas *et al.*, 1994). Higher moisture content in the soil should therefore reduce the available O_2 and stimulate higher denitrification rates, and subsequently N_2O-N production. This tendency can be seen in Figure 5.5.

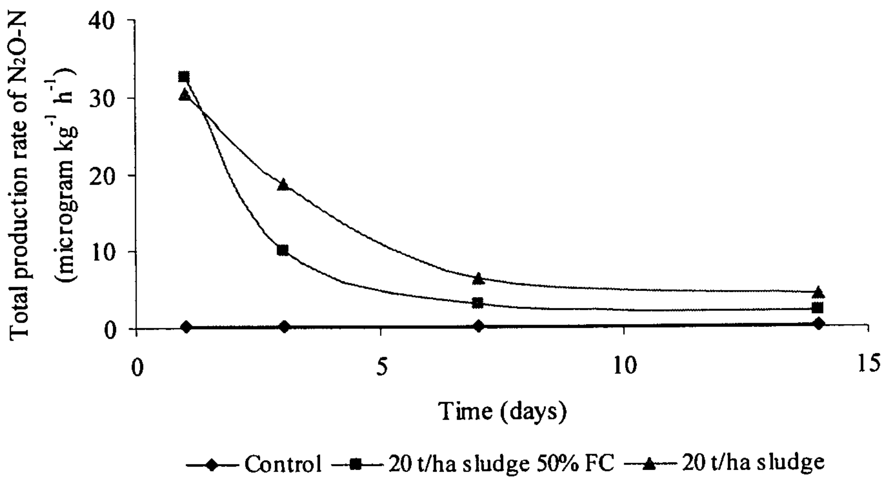


Figure 5.5: The influence of moisture content on N_2O-N emission

According to Figure 5.5, the initial rate of N_2O-N production is the same, but the production rate in the 50% FC treatment decreases faster than the 100% FC treatment. The total amount of N_2O-N released will be higher from the 100% FC treatment than from 50% FC. Although the N_2O production rate from wetter soil was higher, the difference between the wetter and drier soil were not significant. These results were in line with results in a similar study done by Webster & Hopkins (1996). They found that the production of N_2O was greater in the wetter compared to the drier soil, but because the consumption of N_2O by denitrifiers was also greater in the wetter soil, the actual emissions rates were similar.

5.3.3. Fertiliser treatments

According to Vermoesen *et al.* (1996), the production of N_2O and NO is positively related to the inorganic N availability. In contrast to this statement, the fertiliser treatments in this experiment did not show any significant differences from the control treatment (Figure 5.6).

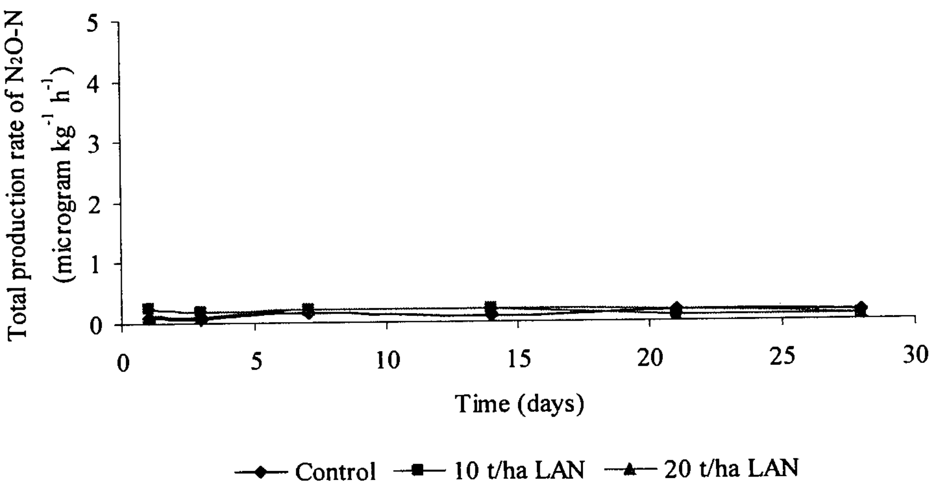


Figure 5.6: The total production rate of N_2O -N in µg per kg dry soil per hour over the extent of 28 days for the fertilizer treatment

A possible reason for this could be the lack of organic matter. N_2O -N is always released during denitrification, and most denitrification in soil is associated with organic matter deposits (Coyne, 1999). The absence of organic matter could inhibit the microorganism activity, which in turn result in low N_2O -N production. According to studies done by Bosatta & Agren, (1995), addition of inorganic fertilizer to the soil, resulted in no change in microbial activity. However, if inorganic N is available, fungi are capable of increasing their N concentration. Application of sludge though, greatly stimulates microbial activity (Jansson & Persson, 1982), as was also the result in this experiment.

5.4. DISCUSSION AND CONCLUSIONS

Higher applications of sludge resulted in higher N_2O-N formation. During mineralisation of sludge, microorganisms break organically bound N into inorganic components. During this process N_2O is released. If the rate of N_2O formation can be used as an indication of mineralisation rate, it was found that most mineralisation were complete after 21 days. The easily mineralisable components are first utilised rapidly, after which more complex organic substances are utilised. Mineralisation does not cease to take place, it only stabilises, and very low levels of N_2O are released.

Contrary to expectations the inorganic fertiliser did not show any N_2O production. This is not in line with other researchers' findings. Possible reasons for these poor results could be lower microbial activity due to absence of organic material.

Significant differences were obtained from different moisture contents. High moisture content, at 100% FC showed greater N_2O formation than lower moisture contents (50% FC), due to more anaerobic sites in the soil, which favours denitrification.

In practices, the N_2O emissions will be less than in this experiment. The experiment was carried out under ideal conditions, constant moisture content and temperature. The sludge was also applied in a very fine form that increases the contact surface that can lead to more effective mineralisation by the microorganisms. This resulted in apparent higher N_2O formation than will be observed in the field. The opposite could also be true, where N_2O emissions in practice could be higher than in the experiment. The soil used in the experiment was grained and sieved, and therefore did not have any aggregates. Microsites on aggregates can promote denitrification, and increase the lost of N through this pathway.

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CHAPTER 6

N-Mineralisation rate of sewage sludge and N balance equation

6.1. INTRODUCTION

The quantification of the degradation rates of sewage sludge, or any organic material, is important to evaluate the availability and cycling in nature of nutrient such as C, N, S and P. The decomposition rate can be measured by quantifying the change in sewage sludge composition. The decomposition rate is proportional to the concentrations of the reactants raised to a power. The power to which the concentration of a reactant is raised is the reaction order, eg. rate = $k[A]^1$ is a first order reaction. The overall reaction rate is the sum of the individual orders. For the reaction where rate = $k[A]^1[B]^1$ both A and B individually is first order reactions, but the overall reaction is a second order. (Atkins, 1999; Paul & Clark, 1989). Several reactions orders exist, of which the most common reactions are: zero-, first- and second-order reactions.

The aim of the trial was mainly to determine the mineralisation rate of the sludge (and subsequently NO_3^- production rate) by using kinetics reactions. The application of reaction kinetics was proposed by Stanford and Smith (1972), and provides an apparent rate coefficient for the release of N from soils. N- mineralisation from organic substances is best described by using the first-order kinetics (Coyne, 1999; Paul & Clark.1989), as also done in studies by Carski & Sparks, (1987). For this reason, only first order reactions will be discussed.

6.1.1. Mineralisation rates

The rate law for first order mineralisation reaction can be expressed as follows:

$$\frac{dN}{dt} = -kN \qquad (1)$$

Integration of equation 1 gives:

$$N_t = N_o e^{-kt} \qquad (2)$$

where N_t is the N-concentration in sewage sludge that remained at any time t, N_o is the original N content in the sewage sludge and k is a mineralisation rate constant.

The negative sign in the equation is the result of the oxidation of the substrate. The graphic presentation of the N-mineralisation that follows first order kinetics is illustrated in Figure 6.1 (Coyne, 1999; Paul & Clark.1989; Atkins, 1999).

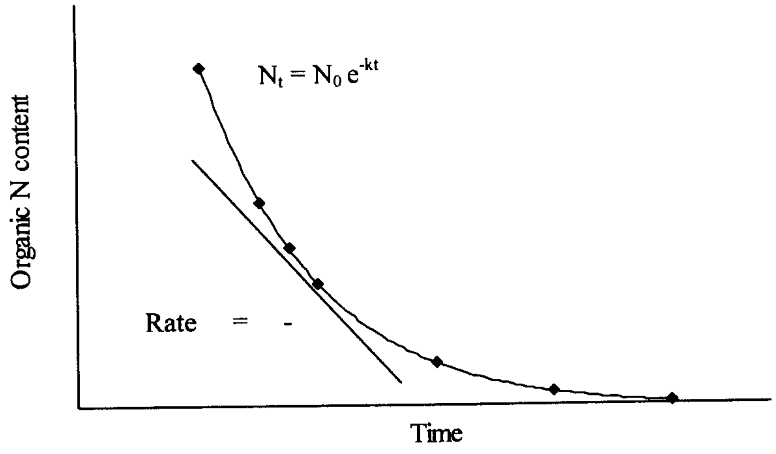

Figure 6.1: Theoretical plot of a fist order N mineralisation reaction

In most N mineralisation studies, the increase in the inorganic N products, such as NH_4^+ and NO_3^-, are measured, rather than the decrease in the N content of the organic matter. The rate at which organic material decomposes, and the rate at which inorganic N forms are released, should be the same. Decomposition has a negative rate constant (Figure 6.1), while the production of inorganic products has a positive rate constant (Figure 6.2). Because it is easier to measure the production of NH_4^+ and NO_3^-, rather than the decrease in sewage sludge, the mineralisation rate in this study will be expressed in terms of the products formed.

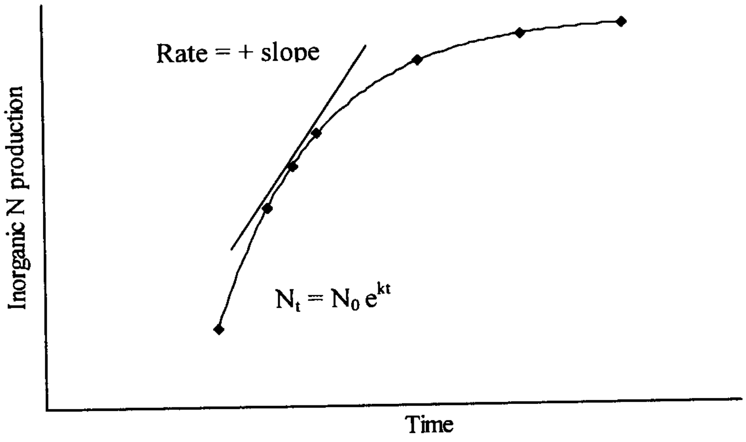

Figure 6.2: Schematic diagram of N-mineralisation of multiple organic N compounds adapted from Coyne (1999)

The fact that the slope of the curve in Figure 6.2 stabilises with time indicates a decrease in the production rate of inorganic N. This also indicates that all the organic N components are not equally mineralised and consequently do not mineralise at the same rates. N forms such as urea, amino acids and proteins are rapidly mineralised, followed by the more stable N forms such as amino sugars and humified N that are mineralised at a slower rate (Coyne, 1999; Carski & Sparks, 1987).

6.1.2. Rate constants

If the rate constants of a kinetic data set, e.g. mineralisation of sewage sludge, is known, the exact rate at different time intervals can be calculated by multiplying the concentration of the remaining N concentration in the sludge with the rate constant.

The rate constant can be expressed by taking the ln (natural logarithm) on both sides of equation 2:

$$\ln(N_t / N_o) = -kt \qquad (3)$$

From this expression it follows that,

$$\ln N_t - \ln N_o = -kt$$
$$\ln N_t = -kt + \ln N_o \qquad (4)$$
$$(y \ = mx + c)$$

The slope of this linear equation gives the rate constant. The rate constant has a fixed value and does not change, whereas the mineralisation rate will constantly change, due to the decrease in the N containing substrate (N_t). The graphic expression of equation 3 is illustrated in Figure 6.3.

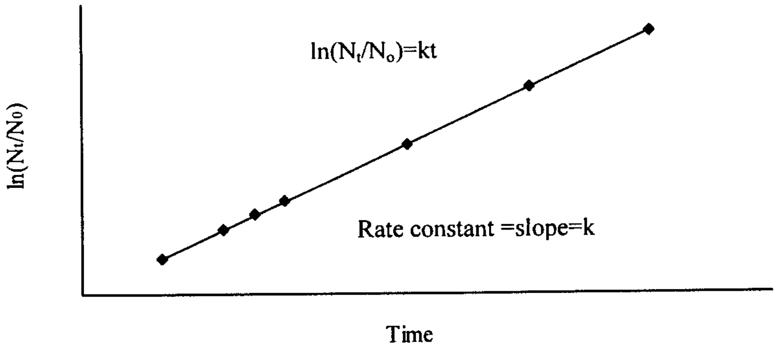

Figure 6.3: Theoretical presentation of a natural logarithmic graph of the rate constant from products formed (same data used in Figure 6.2)

In Figure 6.3 the slope of the linear regression is positive, because it illustrates the formation of products and not the decrease in organic substance. The data in this chapter will be represented in this form.

The mineralisation rate constants of sludge, in terms of NH_4^+ and NO_3^- production, were calculated using equation 3, using the data of the incubation trial (Chapter 3). After integration of the values, straight lines could be fitted on the data set. According to this data, it was clear that two stages in the mineralisation process could be identified. Two linear regressions were fitted to give a regression equation that gave a better correlation coefficient (R^2). The slopes of the lines, k, represents the rate constants per day (also see Figure. 6.3).

6.1.3. N transformations

In this study, the sum of NH_4^+ and NO_3^- production was used to quantify the sewage sludge mineralisation. More accurate mineralisation rates can be obtained by also including the production of N_2O and N_2 etc. However, it was found that the amount of N losses through pathways, other than ammonification and nitrification were very little, and therefore not included in the calculations (see Chapter 6.1.5).

c. *20 ton ha^{-1} treatment*

It was assumed that the change in inorganic N could only be the result of oxidation of the added sludge, and subsequently the mineralisation of sludge. Figure 6.4 shows two distinct mineralisation stages, the first is characterised by a high mineralisation rate (a high NH_4^+-N production, but very low NO_3^- production), and in the second stage nitrification is dominant, while NH_4^+ production decreased.

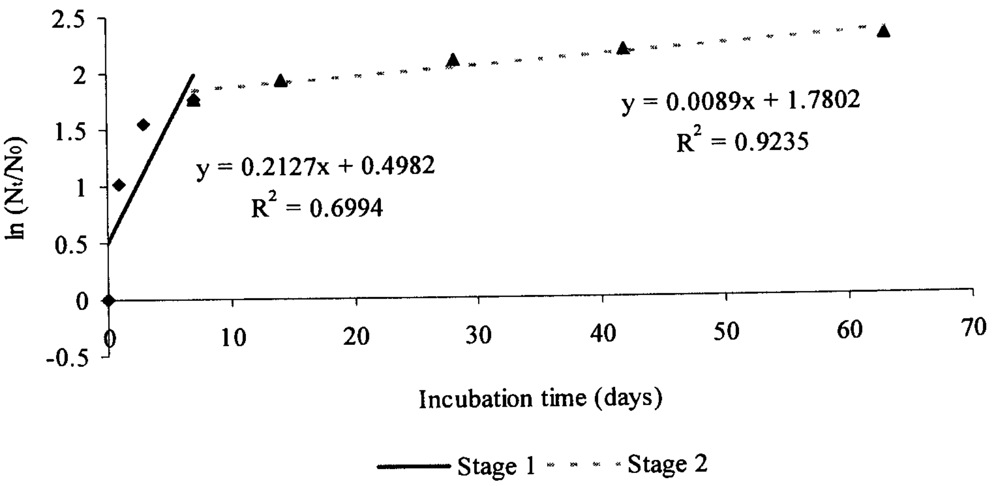

Figure 6.4: Plot of the natural logarithm of the sludge mineralisation rate vs. incubation time at the 20 ton ha^{-1} treatment

For stage 1, the mineralisation constant (slope of the line) is 0.2127 day^{-1}, and decline to 0.0089 day^{-1} in stage 2, that could be due to the decrease in easily mineralisable organic N. This indicates an initial fast mineralisation that decreased after seven days to a more stable and constant mineralisation rate.

d. *10 ton ha^{-1} treatments*

Natural logarithm graphs of the 10 ton ha^{-1} treatments were similar to that of the 20 ton ha^{-1} treatments. It could also be divided into two stages. The total mineralisation rates of the sludge are presented in Figure 6.5.

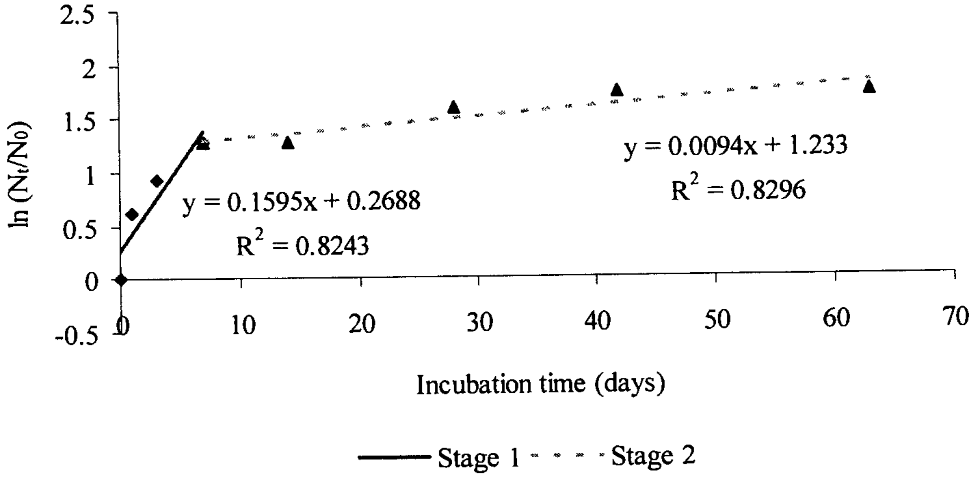

Figure 6.5: Plot of the natural logarithm of the sludge mineralisation rate vs. incubation time at the 10 ton ha^{-1} treatment

The rate constants for the different treatments are summarised in Table 6.1.

Table 6.1: Rate constants (day^{-1}) of the different stages and treatments

	20 ton ha^{-1}	**10 ton ha^{-1}**
Stage 1 (Day 0 – 7)	0.212 day^{-1}	0.160 day^{-1}
Stage 2 (Day 7-62)	0.009 day^{-1}	0.009 day^{-1}

From the mineralisation rate constants at 20 ton ha^{-1}, it can be seen that rapid mineralisation occurs at the first stage with a rate constants of 0.212 day^{-1}, followed by a lower mineralisation rate constants of 0.009 day^{-1}. The same tendency occurs at the 10ton ha^{-1} application rate, with a rate constant of 0.16 day^{-1} for stage 1, and also 0.009 day^{-1} for stage 2. This indicates that mineralisation rate initially increases with an increase in sludge application, but after 7 days, the mineralisation rate constants are similar.

6.1.4. Mineralisation rates

Mineralisation rates can be obtained by multiplying the rate constants with the concentration of the products. The rate at which the mineralisation takes place at that specific moment can be calculated accordingly. These rate constants can be applied to similar sludges, to obtain the rate at which the sludge will be mineralised at similar conditions (moisture content, soil types and temperatures). For this specific trial, the mineralisation rate of the 20 ton ha^{-1} treatment sewage sludge were calculated and presented in Table 6.2.

Table 6.2: Calculated mineralisation rate constants of sewage sludge, according to the daily production of NO_3^- and NH_4^+

Rate constants				**Rate constant: 0.0009 day^{-1}**				
	Rate constant: 0.212 day^{-1}							
Days	0	1	3	7	14	28	42	63
Cumulative production of NO_3^- and NH_4^+*	11.98	33.28	56.15	70.26	82.81	97.27	106.79	119.24
Daily production (mg kg^{-1} day^{-1})	0.00	21.30	11.43	3.53	1.79	1.03	0.68	0.59
Rates constants: (day^{-1})		4.52	2.42	0.75	0.02	0.01	0.01	0.01

*Measured in mg/kg

The total cumulative values and the daily production of inorganic N in terms of NH_4^+ and NO_3^- release in the 20 ton ha^{-1} treatment is given. The mineralisation rate reaction for every set is also given in Table 6.2. There are overlaps in the rates on day 7, due to the common points that are shared in the linear regression. The mineralisation rate on day 7 was calculated with the first rate constant (0.212 day^{-1}).

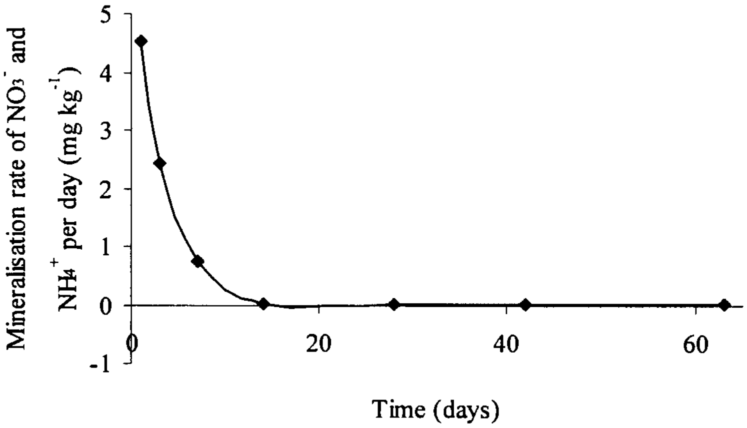

Figure 6.6: Mineralisation rate in mg kg^{-1} day^{-1} in terms of the total inorganic N (NO_3^- and NH_4^+) production, as influenced by incubation days.

From the results in Figure 6.6 it can be concluded that the mineralisation rate initially starts at a maximum on day 1 and decreases up to day 14 where it remains low and steady. This implies that during the first 14 days, most of the mineralisation will take place and be completed. The actual rate at which organic material will be broken down in the soil, declines over time.

6.1.5. N balance equation

The cumulative fractions of the different inorganic N forms over time are not constant. This is because of the transformation of NH_4^+ to NO_3^-; change in mineralisation rate; N losses through denitrification and ammonia volatilisation. It is therefore not possible to give a single N equation, or balance. It must be linked with time for a continuous evaluation of change.

i. Sewage sludge

The remaining organic N fraction of the sludge in the soil was theoretically calculated by subtracting the extractable inorganic fractions from the total original N content of the sludge. Although this is not totally accurate, it gives an opportunity to calculate N balance. In general it is very difficult to account for all the N in a system, and the imbalance between inputs and outputs are experienced by many researches over the years (Wrage *et al.*, 2001).

In Figure 6.7, the contents of the different N fractions measured during the different incubation studies are given. The values are in terms of cumulative production, and not daily production.

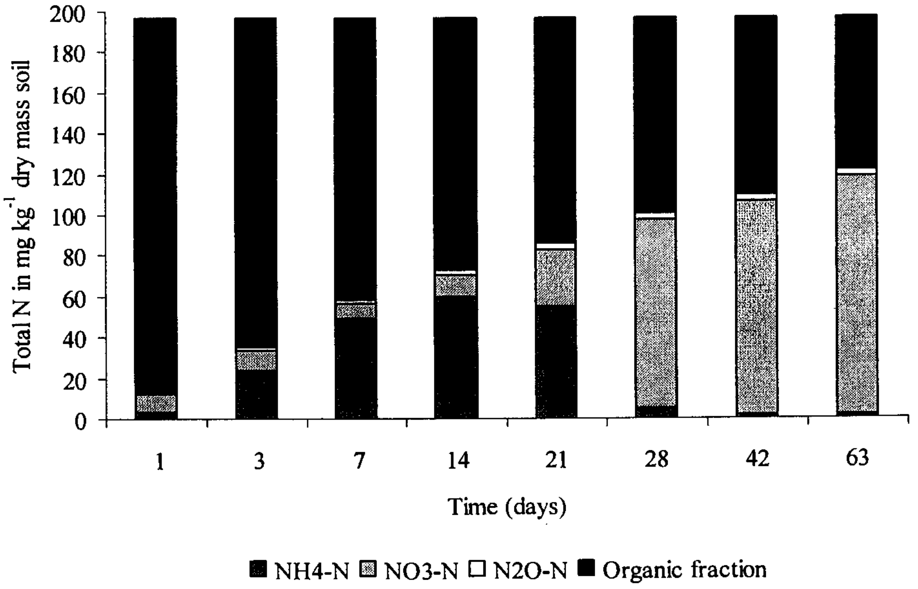

Figure 6.7: Cumulative change of different N fractions over time in the 20 ton ha^{-1} sludge treatment

Figure 6.7 shows that one day after application of the sewage sludge, only 6.4% of the N is inorganic which was mainly in the form of NO_3^-. The NH_4^+ content increase rapidly, as the sludge is being mineralised by microorganism, and decreases again due to nitrification. The cumulative fractions of N2O were relatively low compared to the other fractions. After 63 days only 37.8% of the organic N remained and probably consisted of more complex organic matter that is more stable. The inorganic fraction after 63 days, was 62.2% of the total N of which the largest inorganic fraction present was NO_3^-, which accounted for 60% of the total N. The remaining inorganic N consisted of N_2O emissions (1.6 %) and NH_4^+ (0.7%). This findings is not in line with the 'Permissible Utilisation and Disposal of Sewage Sludge', which states that only 30% of the N sludge becomes available in the first year, 15% in the second and 5% in the third year (WRC, 1997).

At lower application rates such as 10 ton ha^{-1} sludge, the results were much similar to that of the 20 ton ha^{-1} application (Figure 6.8).

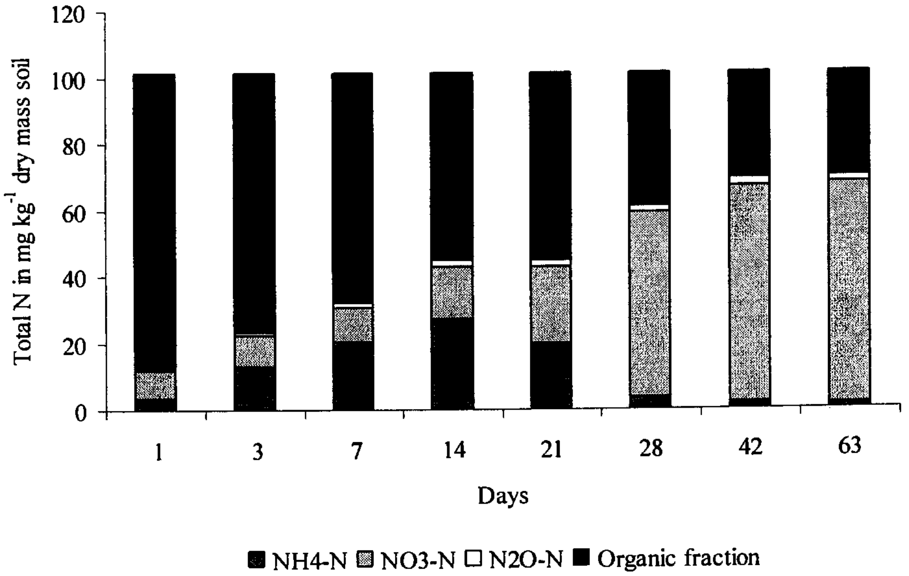

Figure 6.8: Cumulative change of different inorganic N fractions over time in the 10 ton ha^{-1} sludge treatment

Although a similar trend was obtained, the absolute values were lower by about half, as can be expected, but the ratios of different N forms did not differ much. At day 1, the percentage organic matter in terms of N content, was 87.7%, and the inorganic fraction only 12.3%. This value changed during the 63 days to 30.8% organic and 69.2% inorganic N. The relative higher inorganic N content can be ascribed to the natural N content of the soil, which is percentage wise higher in the 10 ton ha^{-1} compared to the 20 ton ha^{-1} treatment.

ii. Fertilizer treatment

The absence of organic matter in the fertilizer treatments makes the N balance much less complicated. The original N applied in the form of LAN fertilizer, as well as the different fractions measured in the different incubation studies were used to calculate the N balances. The N balances in the equivalent of the 20 ton ha^{-1} and 10 ton ha^{-1} sludge treatments are illustrated in Figure 6.9 and 6.10 respectively.

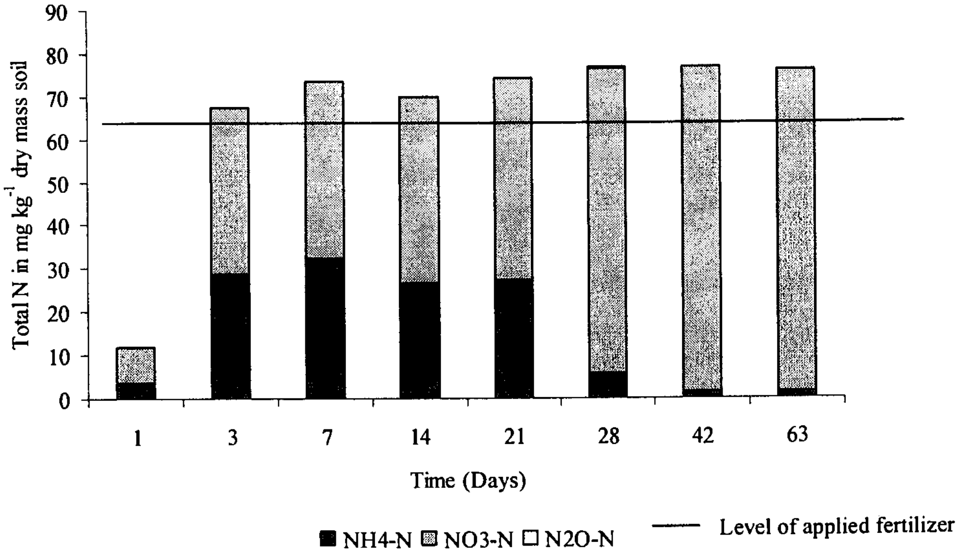

Figure 6.9: Cumulative change of different inorganic N fractions over time in the "20 ton ha^{-1}" fertilizer treatment

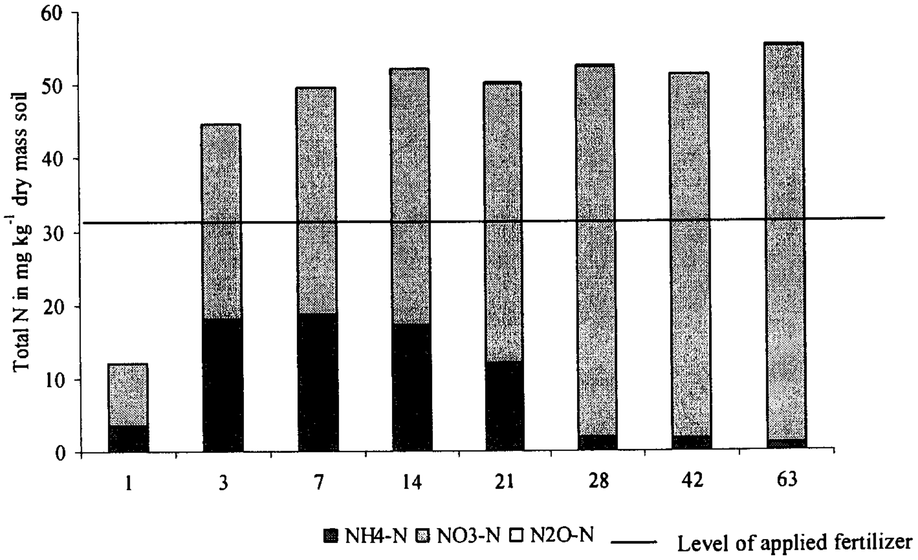

Figure 6.10: Cumulative change of different inorganic N fractions over time in the "10 ton ha^{-1}" fertilizer treatment

Figure 6.9 and 6.10 show that all the fertilizer had been available in the inorganic form from day 3 onwards. Similar to the sludge treatments, the NH_4^+ and NO_3^- fractions changed over time as a result of nitrification. In the fertilizer treatments, no N_2O was measured. The level of applied fertilizer (indicated with a line in figure 6.9 and 6.10) indicates the theoretical quantity of fertilizer applied in the experiment. This fertilizer fraction indicates that more inorganic N was release as was applied, according to the calculations. This can be the result of experimental errors due to the small quantities of fertilizers used in the incubation trials. It would be easy to over estimate the application levels. The error of overestimation is even bigger in the 10 ton ha^{-1} treatment than in the 20 ton ha^{-1} treatment, which also confirms the possibility of inaccuracy during the experimental proceedings.

6.2. CONCLUSIONS

According to kinetic calculations, and previous experiments, it can be concluded that there is a correlation between the mineralisation of sewage sludge and release of inorganic N, especially NO_3^-. Higher sludge application leads to an increase in mineralisation rates and subsequently more inorganic N release. This mineralisation rate can be divided into two stages with different rate constants: stage 1 (day 0-7) and stage 2 (day 7-63+). Mineralisation rates was calculated according to the rate constants, and showed that mineralisation was initially fast, but decreased with time. Initially NH_4^+ production dominated the inorganic N rates. After 7 days this situation changes when NH_4^+ production declined, and NO_3^- production increased.

After the duration of the experiments (63 days), 62.2% of the applied N sewage sludge has been mineralised into inorganic N, mainly in the form of NO_3^- ions (60%), but also N_2O gas (1.6%). It seems as if large quantities of inorganic N becomes available for groundwater contaminations, as well as N_2O emissions that contribute to greenhouse gasses. However, these assumptions are made on laboratory experiments, under ideal circumstances. In practices, the mineralisation rate will be delayed by environmental factors, such as variation in moisture content, consumption by plants, temperature change, different soil types etc. These values are therefore the worse case scenario, and the effect, especially in the presence of plants would be much less.

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CHAPTER 7

Conclusions and Recommendations

7.1. CONCLUSIONS AND RECOMMENDATIONS

The experiments in this study were designed to measure all the different forms of inorganic N that are produced in the soil, after application of sewage sludge and commercial fertilizer. In a first laboratory trial, NH_4^+ and NO_3^- production were measured, and in a second trial N_2O production measured. A third trial was conducted to test the validity of the laboratory results in the field. The main focus of all these trials were to test the possibility of NO_3^- leaching from sewage sludge and commercial fertilizer, and the possible risk of groundwater contamination.

The NH_4^+ fraction in the soil increased immediately after application of the sewage sludge, due to the stimulation in microbial activity. The NH_4^+ production peaked at day 7, after which it declined until all NH_4^+ ions were utilised by micoorganisms, and the production became similar to the control after day 28. Concurrently, the production of NO_3^- increased steadily over time, until it reached a maximum at day 28, after which it stabilised. The same was found for the treatments that received commercial fertilzer that contained NH_4^+ and NO_3^-. The different application rates, 5, 10 and 20 ton ha^{-1}, were compared to similar rates of commercial fertilizer applications. At low application levels, 5 ton ha^{-1}, no significant differences in terms of NH_4^+ production occur between the fertilizer and the sludge treatments, but at the higher application levels, the sludge produced significantly more NH_4^+ than the applied NH_4^+ in fertilizer.

The total NO_3^- produced in the 20 ton ha^{-1} sludge treatment after 63 days was 117.8 mg kg^{-1}, while in the fertilizer treatment, it was 74.6 mg kg^{-1}. This situation looks different if it is taken into account that the total production of NO_3^- from commercial fertilizer was higher than that of sludge until day 28. After this the continuous NO_3^- production in the sludge treatment overtakes the total production from that of the fertilizer. This could be ascribed to the fact that more of the organic N in the sludge was mineralised than expected. These expectations were based on data from the literature. At the lower application rates, the values were lower but the trend stayed the same. More important than total production of the NH_4^+ and the NO_3^- is the distribution and availability of NO_3^- production over time. In the sludge treatment, the maximum NO_3^- production was only after 28 days due to nitrification of the NH_4^+. In the commercial fertilizer treatment the applied NO_3^- was immediately present after application, but also increased due to nitrification. A more constant production of inorganic N, like in the case of sludge, will have more advantages to crops, which continuously consume nutrients.

In a summer and winter field trial, similar treatments were applied to compare the mineralisation rate under field conditions. Vastly different results were obtained. For the winter trial, no change in NH_4^+ content at different depths were observed. Also no significant changes in NO_3^- content was measured, except for a slight increase in the NO_3^- level after 77 days in the 16 ton ha^{-1} treatment. This could possibly be due to low temperatures and low moisture contents in the winter and subsequently low microbial activity. In the summer trial crops and fallow conditions were also taken into consideration. The NH_4^+ in the 0-30 cm soil layer, showed an immediate increase after application of all treatments. Day 7, on which the first samples were taken, represents the peak in NH_4^+ in the soil. After this peak, the NH_4^+ decreases steadily, but at the end of the experiment, (90 days) NH_4^+ could still be detected in the 0-30 cm soil layer, for all the treatments. Compared to the laboratory experimental data, there was a huge delay in the mineralisation of N in the field experiment. In the laboratory NH_4^+ depletions took only 28 days, whereas in the field, the NH_4^+ was not even totally utilised after 90 days. Crops could also help to reduce the available inorganic N in the soil, by utilising a part of the N. In this study, no significant differences between crops and fallow conditions were found, but other studies did proof that crops will reduce the risk of leaching significantly. Commercial fertilizer on the other hand, could increase the risk of NO_3^- leaching, due to the large fraction of NO_3^- available at one time.

The NO_3^- value for the fertilizer treatment in the field trial showed immediate increases in the 0-30 cm soil layer, after which it steadily decreased. The NO_3^- production in the sludge treatments remained low and constant. In the 30-60 cm soil layer, large amounts of NO_3^- movement occurred in the fertilizer treatment, as the access amounts of NO_3^- were being leached through the profile. This value reached a maximum on day 56, after which it started to decrease again, as the NO_3^- was being removed from the 30-60 cm layer to the 60-90 cm soil layer. The values for the sludge treatments remained constant. In the 60-90 cm soil layer, the only detectible change was on day 90 in the fertilizer treatment when the fraction of NO_3^- from the above soil layer was leached into the lower sub soil. During the 90 days of the experiment, no NO_3^- from the sludge treatments reached the 60-90 cm soil layers.

According to field experiments, the leaching potential for commercial fertilizer, applied at the same N rates than that of sewage sludge, is more likely to leach and cause groundwater contamination than sewage sludge. Unlike expected after results from the laboratory experiment, it took much longer for the sludge to mineralise under field conditions. Low temperature and moisture contents, or fluctuations of these conditions in the field could be responsible for the decrease in mineralisation rate, especially in the winter.

In a lab trial N losses through denitrification (N_2O and N_2 losses) were also measured at two different moisture regimes. All though for the sludge treatments, the values of N_2O emission from the wetter soil was continuously higher than that of the drier soil no significant differences were found between the different moisture contents. Significant differences were found between the control, 10 ton ha^{-1} sludge and 20 ton ha^{-1} sludge treatments.

The total emission rate of N_2O at day 1 was 30.3 and 21.0 µg kg^{-1} dry matter h^{-1} for the 20 and 10 ton ha^{-1} treatment respectively, but declined after 7 days 6.4 and 2.9µg kg^{-1} dry matter h^{-1} respectively. After 7 days the emission further decreases until it reaches an insignificant level at day 21. In this study, the fertilizer treatments did not differ significantly from the control. This could be due to low organic matter content in the fertilizer treatment, and subsequently no stimulation of heterotrophic microorganisms.

According to the kinetic data the rate constant of mineralisation is dependent on time. The mineralisation rate constant can be divided into two stages: stage 1 (day 0-7) and stage 2 (day 7- 63). Stage one has a high rate constant, while the rate constant is much lower in stage 2. The mineralisation rates that were calculated according to the rate constants also show a high mineralisation rate during the beginning of the trial, whereas the rate decreased over time. These mineralisation rate constants give an indication of what could happen to sewage sludge that is applied to agricultural soil. These values are constant, and independent of the specific sludge used in these experiments. By multiplying the rate constants with the concentration of organic N of each specific sludge, the theoretical mineralisation rate over time can be calculated for different sludges in different circumstances. The amount of NO_3^- release could be estimated. It should be kept in mind that this is not totally accurate, because aspects like climate, soil type, irrigation, method of application etc., will also play a role in the mineralisation of sludge.

After all the inorganic fractions of N were measured, the different fractions of N, organic and inorganic were calculated over time. According to these calculations, between 6 and 12 % of the N is available after one day from sludge application. This value changes after 63 days to the availability of 62 to 70% of inorganic N. Of this fraction NO_3^- is by far the most abundant product with N_2O and NH4 in limited amounts.

In conclusion it can be said that sewage sludge can act as a slow-release fertilizer that will supply constant NH_4^+ and NO_3^- to plants for a longer period. This will mean a more effective utilisation of applied N with lower risk for NO_3^- leaching. Contrary to the commercial fertilizer, especially NO_3^- application at any time could be more prone to leaching. At high levels of sludge application, such as 20 ton dried sludge ha^{-1} $year^{-1}$, the total production of NO_3^- will be greater than that of an equivalent fertilizer treatment, but the distribution through the crops growing season will be more gradual and constant.

The current legal application rate of 8 ton ha^{-1} dried sludge $year^{-1}$, is found to be very low, and application levels of up to 20 ton ha^{-1} $year^{-1}$ did not show any signs of NO_3^- leaching. This study alone is however not enough to base important decisions on. All the aspects must be taken into account, and if possible a model for NO_3^- leaching must be developed where soil type, temperature, rainfall, irrigation, type of sludge, etc. could be taken into account.

APPENDIX 1:

STATISTICAL ANALYSIS OF INCUBATION TRIAL

Incubation trial

Abbreviations

Beh: Behandeling (Treatment)
Per: Periode (Period; Time intervals)
Her: Herhaling (Repetition)

Statistical numbers		Corresponding experimental treatments
Beh	1	5 ton/ha sludge
	2	10 ton/ha sludge
	3	20 ton/ha sludge
	4	5 ton/ha fertilizer
	5	10 ton/ha fertilizer
	7	20 ton/ha fertilizer
	8	Control

Statistical numbers		Corresponding experimental days
Per	1	0
	2	1
	3	3
	4	7
	5	14
	6	28
	7	42
	8	63

```
                    N INKUBASIE STATS NH4              10:26 Tuesday, September 2, 2003   1

                              The GLM Procedure
                           Class Level Information
                         Class        Levels    Values
                       BEHAN              7    1 2 3 4 5 6 7
                       PER              8    1 2 3 4 5 6 7 8


                       Number of observations    168
             1N INKUBASIE STATS NH4              10:26 Tuesday, September 2, 2003   2

                              The GLM Procedure
                       Dependent Variable: V1   NH4

Class Level Information


          Class                     Levels                    Values
          BEHAN                 7                          1 2 3 4 5 6 7
                 0.983045     20.30833     2.179712     10.73310
```

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BEHAN	6	8380.48237	1396.74706	293.98	<.0001
PER	7	12426.73245	1775.24749	373.65	<.0001
BEHAN*PER	42	10044.47750	239.15423	50.34	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
BEHAN	6	8380.48237	1396.74706	293.98	<.0001
PER	7	12426.73245	1775.24749	373.65	<.0001
BEHAN*PER	42	10044.47750	239.15423	50.34	<.0001

The GLM Procedure, Least Squares Means

BEHAN	Standard V1 LSMEAN	LSMEAN Error	Pr > |t|	Number
1	6.1845833	0.4449319	<.0001	1
2	11.2225000	0.4449319	<.0001	2
3	24.8762500	0.4449319	<.0001	3
4	5.3612500	0.4449319	<.0001	4
5	9.2862500	0.4449319	<.0001	5
6	15.8808333	0.4449319	<.0001	6
7	2.3200000	0.4449319	<.0001	7

Least Squares Means for effect BEHAN

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: V1

i/j	1	2	3	4	5	6	7
1		<.0001	<.0001	0.1934	<.0001	<.0001	<.0001
2	<.0001		<.0001	<.0001	0.0026	<.0001	<.0001
3	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
4	0.1934	<.0001	<.0001		<.0001	<.0001	<.0001
5	<.0001	0.0026	<.0001	<.0001		<.0001	<.0001
6	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
7	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	

Class	Levels	Values
BEHAN	7	1 2 3 4 5 6 7

4	22.5300000	0.4756522	<.0001	4
5	18.6223810	0.4756522	<.0001	5
6	2.9690476	0.4756522	<.0001	6
7	1.7361905	0.4756522	0.0004	7
8	1.3309524	0.4756522	0.0061	8

```
                         Least Squares Means for effect PER
                        Pr > |t| for H0: LSMean(i)=LSMean(j)
                              Dependent Variable: V1

i/j       1         2         3         4         5         6         7         8

1                  <.0001    <.0001    <.0001    <.0001    0.4089    0.0089    0.0015
2       <.0001               <.0001    <.0001    <.0001    <.0001    <.0001    <.0001
3       <.0001    <.0001               0.0014    0.0126    <.0001    <.0001    <.0001
4       <.0001    <.0001    0.0014               <.0001    <.0001    <.0001    <.0001
5       <.0001    <.0001    0.0126    <.0001               <.0001    <.0001    <.0001
6       0.4089    <.0001    <.0001    <.0001    <.0001               0.0695    0.0165
7       0.0089    <.0001    <.0001    <.0001    <.0001    0.0695              0.5481
8       0.0015    <.0001    <.0001    <.0001    <.0001    0.0165    0.5481

                       Standard              LSMEAN
BEHAN   PER    V1 LSMEAN        Error   Pr > |t|    Number

1       1      3.5266667    1.2584574   0.0060      1
1       2      8.1033333    1.2584574   <.0001      2
1       3     10.6800000    1.2584574   <.0001      3
1       4     13.5900000    1.2584574   <.0001      4
```

1	5	9.0966667	1.2584574	<.0001	5
1	6	1.7733333	1.2584574	0.1616	6
1	7	1.3433333	1.2584574	0.2881	7
1	8	1.3633333	1.2584574	0.2810	8
2	1	3.5266667	1.2584574	0.0060	9
2	2	12.7100000	1.2584574	<.0001	10
2	3	20.0100000	1.2584574	<.0001	11
2	4	27.2166667	1.2584574	<.0001	12
2	5	19.5633333	1.2584574	<.0001	13
2	6	3.5466667	1.2584574	0.0057	14
2	7	1.8600000	1.2584574	0.1422	15
2	8	1.3466667	1.2584574	0.2869	16
3	1	3.5266667	1.2584574	0.0060	17
3	2	23.6500000	1.2584574	<.0001	18
3	3	49.4666667	1.2584574	<.0001	19
3	4	59.8433333	1.2584574	<.0001	20
3	5	55.1033333	1.2584574	<.0001	21
3	6	4.5433333	1.2584574	0.0005	22
3	7	1.4566667	1.2584574	0.2495	23
3	8	1.4200000	1.2584574	0.2616	24
4	1	3.5266667	1.2584574	0.0060	25
4	2	9.7633333	1.2584574	<.0001	26

```
4     3     8.8500000     1.2584574    <.0001      27
4     4    11.0333333     1.2584574    <.0001      28
4     5     4.6833333     1.2584574    0.0003      29
4     6     2.5333333     1.2584574    0.0465      30
4     7     1.2333333     1.2584574    0.3292      31
4     8     1.2666667     1.2584574    0.3163      32

              The GLM Procedure, Least Squares Means

                            Standard        LSMEAN
BEHAN   PER   V1 LSMEAN       Error    Pr > |t|     Number

5     1     3.5266667     1.2584574    0.0060      33
5     2    18.1600000     1.2584574    <.0001      34
5     3    18.6666667     1.2584574    <.0001      35
5     4    17.2666667     1.2584574    <.0001      36
5     5    11.9666667     1.2584574    <.0001      37
5     6     1.9033333     1.2584574    0.1332      38
5     7     1.6600000     1.2584574    0.1898      39
5     8     1.1400000     1.2584574    0.3669      40
6     1     3.5266667     1.2584574    0.0060      41
6     2    28.6900000     1.2584574    <.0001      42
```

```
6   3   32.3300000   1.2584574   <.0001   43
6   4   26.5433333   1.2584574   <.0001   44
6   5   27.1966667   1.2584574   <.0001   45
6   6    5.8866667   1.2584574   <.0001   46
6   7    1.5500000   1.2584574   0.2207   47
6   8    1.3233333   1.2584574   0.2953   48
7   1    3.5266667   1.2584574   0.0060   49
7   2    2.6700000   1.2584574   0.0361   50
7   3    2.2966667   1.2584574   0.0707   51
7   4    2.2166667   1.2584574   0.0809   52
7   5    2.7466667   1.2584574   0.0312   53
7   6    0.5966667   1.2584574   0.6363   54
7   7    3.0500000   1.2584574   0.0170   55
7   8    1.4566667   1.2584574   0.2495   56


                          The GLM Procedure
                         Least Squares Means

                Least Squares Means for effect BEHAN*PER
                 Pr > |t| for H0: LSMean(i)=LSMean(j)
                       Dependent Variable: V1
```

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		0.0114	0.0001	<.0001	0.0022	0.3267	0.2225	0.2267	1.0000	<.0001	<.0001	<.0001	<.0001	0.9911
2	0.0114		0.1505	0.0026	0.5779	0.0006	0.0002	0.0002	0.0114	0.0109	<.0001	<.0001	<.0001	0.0118
3	0.0001	0.1505		0.1048	0.3756	<.0001	<.0001	<.0001	0.0001	0.2565	<.0001	<.0001	<.0001	0.0001
4	<.0001	0.0026	0.1048		0.0130	<.0001	<.0001	<.0001	<.0001	0.6220	0.0005	<.0001	0.0011	<.0001
5	0.0022	0.5779	0.3756	0.0130		<.0001	<.0001	<.0001	0.0022	0.0447	<.0001	<.0001	<.0001	0.0023
6	0.3267	0.0006	<.0001	<.0001	<.0001		0.8095	0.8182	0.3267	<.0001	<.0001	<.0001	<.0001	0.3212
7	0.2225	0.0002	<.0001	<.0001	<.0001	0.8095		0.9911	0.2225	<.0001	<.0001	<.0001	<.0001	0.2183
8	0.2267	0.0002	<.0001	<.0001	<.0001	0.8182	0.9911		0.2267	<.0001	<.0001	<.0001	<.0001	0.2225
9	1.0000	0.0114	0.0001	<.0001	0.0022	0.3267	0.2225	0.2267		<.0001	<.0001	<.0001	<.0001	0.9911
10	<.0001	0.0109	0.2565	0.6220	0.0447	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	0.0002	<.0001
11	<.0001	<.0001	<.0001	0.0005	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	0.8023	<.0001
12	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
13	<.0001	<.0001	<.0001	0.0011	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	0.8023	<.0001		<.0001
14	0.9911	0.0118	0.0001	<.0001	0.0023	0.3212	0.2183	0.2225	0.9911	<.0001	<.0001	<.0001	<.0001	
15	0.3510	0.0007	<.0001	<.0001	<.0001	0.9612	0.7721	0.7807	0.3510	<.0001	<.0001	<.0001	<.0001	0.3453
16	0.2232	0.0002	<.0001	<.0001	<.0001	0.8110	0.9985	0.9925	0.2232	<.0001	<.0001	<.0001	<.0001	0.2190
17	1.0000	0.0114	0.0001	<.0001	0.0022	0.3267	0.2225	0.2267	1.0000	<.0001	<.0001	<.0001	<.0001	0.9911
18	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0432	0.0475	0.0235	<.0001
19	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

20		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
21		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
22		0.5690	0.0479	0.0008	<.0001	0.0118	0.1224	0.0749	0.0767	0.5690	<.0001	<.0001	<.0001	<.0001	0.5766
23		0.2473	0.0003	<.0001	<.0001	<.0001	0.8591	0.9493	0.9583	0.2473	<.0001	<.0001	<.0001	<.0001	0.2428
24		0.2390	0.0003	<.0001	<.0001	<.0001	0.8430	0.9657	0.9747	0.2390	<.0001	<.0001	<.0001	<.0001	0.2346
25		1.0000	0.0114	0.0001	<.0001	0.0022	0.3267	0.2225	0.2267	1.0000	<.0001	<.0001	<.0001	<.0001	0.9911
26		0.0007	0.3530	0.6075	0.0337	0.7087	<.0001	<.0001	<.0001	0.0007	0.1006	<.0001	<.0001	<.0001	0.0007
27		0.0034	0.6756	0.3060	0.0089	0.8900	0.0001	<.0001	<.0001	0.0034	0.0322	<.0001	<.0001	<.0001	0.0035
28		<.0001	0.1025	0.8430	0.1536	0.2789	<.0001	<.0001	<.0001	<.0001	0.3482	<.0001	<.0001	<.0001	<.0001
29	0.5171	0.0572	0.0010	<.0001	0.0146	0.1048	0.0632	0.0647	0.5171	<.0001	<.0001	<.0001	<.0001	0.5243	
30	0.5779	0.0022	<.0001	<.0001	0.0004	0.6702	0.5051	0.5123	0.5779	<.0001	<.0001	<.0001	<.0001	0.5702	
31	0.2002	0.0002	<.0001	<.0001	<.0001	0.7621	0.9508	0.9419	0.2002	<.0001	<.0001	<.0001	<.0001	0.1963	
32	0.2068	0.0002	<.0001	<.0001	<.0001	0.7764	0.9657	0.9568	0.2068	<.0001	<.0001	<.0001	<.0001	0.2028	
33	1.0000	0.0114	0.0001	<.0001	0.0022	0.3267	0.2225	0.2267	1.0000	<.0001	<.0001	<.0001	<.0001	0.9911	
34	<.0001	<.0001	<.0001	0.0116	<.0001	<.0001	<.0001	<.0001	<.0001	0.0028	0.3008	<.0001	0.4321	<.0001	
35	<.0001	<.0001	<.0001	0.0052	<.0001	<.0001	<.0001	<.0001	<.0001	0.0011	0.4520	<.0001	0.6154	<.0001	
36	<.0001	<.0001	0.0003	0.0411	<.0001	<.0001	<.0001	<.0001	<.0001	0.0118	0.1260	<.0001	0.1995	<.0001	
37	<.0001	0.0321	0.4712	0.3637	0.1096	<.0001	<.0001	<.0001	<.0001	0.6770	<.0001	<.0001	<.0001	<.0001	
38	0.3637	0.0007	<.0001	<.0001	<.0001	0.9419	0.7536	0.7621	0.3637	<.0001	<.0001	<.0001	<.0001	0.3578	
39	0.2965	0.0004	<.0001	<.0001	<.0001	0.9493	0.8591	0.8679	0.2965	<.0001	<.0001	<.0001	<.0001	0.2914	
40	0.1826	0.0002	<.0001	<.0001	<.0001	0.7226	0.9092	0.9004	0.1826	<.0001	<.0001	<.0001	<.0001	0.1790	
41	1.0000	0.0114	0.0001	<.0001	0.0022	0.3267	0.2225	0.2267	1.0000	<.0001	<.0001	<.0001	<.0001	0.9911	

42	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.4095	<.0001	<.0001
43	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0049	<.0001	<.0001
44	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004	0.7059	0.0002	<.0001
45	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.9911	<.0001	<.0001
46	0.1875	0.2155	0.0082	<.0001	0.0740	0.0227	0.0120	0.0124	0.1875	0.0002	<.0001	<.0001	<.0001	0.1913
47	0.2691	0.0004	<.0001	<.0001	<.0001	0.9004	0.9078	0.9167	0.2691	<.0001	<.0001	<.0001	<.0001	0.2643
48	0.2183	0.0002	<.0001	<.0001	<.0001	0.8008	0.9911	0.9821	0.2183	<.0001	<.0001	<.0001	<.0001	0.2142

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	0.3510	0.2232	1.0000	<.0001	<.0001	<.0001	<.0001	0.5690	0.2473	0.2390	1.0000	0.0007	0.0034	<.0001
2	0.0007	0.0002	0.0114	<.0001	<.0001	<.0001	<.0001	0.0479	0.0003	0.0003	0.0114	0.3530	0.6756	0.1025
3	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001	0.0008	<.0001	<.0001	0.0001	0.6075	0.3060	0.8430
4	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0337	0.0089	0.1536
5	<.0001	<.0001	0.0022	<.0001	<.0001	<.0001	<.0001	0.0118	<.0001	<.0001	0.0022	0.7087	0.8900	0.2789
6	0.9612	0.8110	0.3267	<.0001	<.0001	<.0001	<.0001	0.1224	0.8591	0.8430	0.3267	<.0001	0.0001	<.0001
7	0.7721	0.9985	0.2225	<.0001	<.0001	<.0001	<.0001	0.0749	0.9493	0.9657	0.2225	<.0001	<.0001	<.0001
8	0.7807	0.9925	0.2267	<.0001	<.0001	<.0001	<.0001	0.0767	0.9583	0.9747	0.2267	<.0001	<.0001	<.0001
13	<.0001	<.0001	<.0001	0.0235	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
14	0.3453	0.2190	0.9911	<.0001	<.0001	<.0001	<.0001	0.5766	0.2428	0.2346	0.9911	0.0007	0.0035	<.0001
15		0.7735	0.3510	<.0001	<.0001	<.0001	<.0001	0.1344	0.8211	0.8052	0.3510	<.0001	0.0001	<.0001

38	0.9806	0.7550	0.3637	<.0001	<.0001	<.0001	<.0001	0.1408	0.8023	0.7864	0.3637	<.0001	0.0002	<.0001
39	0.9107	0.8606	0.2965	<.0001	<.0001	<.0001	<.0001	0.1080	0.9092	0.8930	0.2965	<.0001	<.0001	<.0001
40	0.6866	0.9078	0.1826	<.0001	<.0001	<.0001	<.0001	0.0584	0.8591	0.8753	0.1826	<.0001	<.0001	<.0001
41	0.3510	0.2232	1.0000	<.0001	<.0001	<.0001	<.0001	0.5690	0.2473	0.2390	1.0000	0.0007	0.0034	<.0001
42	<.0001	<.0001	<.0001	0.0055	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
43	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
44	<.0001	<.0001	<.0001	0.1068	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
45	<.0001	<.0001	<.0001	0.0487	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
46	0.0256	0.0121	0.1875	<.0001	<.0001	<.0001	<.0001	0.4520	0.0143	0.0135	0.1875	0.0315	0.0987	0.0046
47	0.8620	0.9092	0.2691	<.0001	<.0001	<.0001	<.0001	0.0954	0.9583	0.9419	0.2691	<.0001	<.0001	<.0001
48	0.7636	0.9896	0.2183	<.0001	<.0001	<.0001	<.0001	0.0731	0.9404	0.9568	0.2183	<.0001	<.0001	<.0001

i/j	29	30	31	32	33	34	35	36	37	38	39	40	41	42
1	0.5171	0.5779	0.2002	0.2068	1.0000	<.0001	<.0001	<.0001	<.0001	0.3637	0.2965	0.1826	1.0000	<.0001
2	0.0572	0.0022	0.0002	0.0002	0.0114	<.0001	<.0001	<.0001	0.0321	0.0007	0.0004	0.0002	0.0114	<.0001
3	0.0010	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001	0.0003	0.4712	<.0001	<.0001	<.0001	0.0001	<.0001
4	<.0001	<.0001	<.0001	<.0001	<.0001	0.0116	0.0052	0.0411	0.3637	<.0001	<.0001	<.0001	<.0001	<.0001
5	0.0146	0.0004	<.0001	<.0001	0.0022	<.0001	<.0001	<.0001	0.1096	<.0001	<.0001	<.0001	0.0022	<.0001
6	0.1048	0.6702	0.7621	0.7764	0.3267	<.0001	<.0001	<.0001	<.0001	0.9419	0.9493	0.7226	0.3267	<.0001
7	0.0632	0.5051	0.9508	0.9657	0.2225	<.0001	<.0001	<.0001	<.0001	0.7536	0.8591	0.9092	0.2225	<.0001
8	0.0647	0.5123	0.9419	0.9568	0.2267	<.0001	<.0001	<.0001	<.0001	0.7621	0.8679	0.9004	0.2267	<.0001

9	0.5171	0.5779	0.2002	0.2068	1.0000	<.0001	<.0001	<.0001	<.0001	0.3637	0.2965	0.1826	1.0000	<.0001
10	<.0001	<.0001	<.0001	<.0001	<.0001	0.0028	0.0011	0.0118	0.6770	<.0001	<.0001	<.0001	<.0001	<.0001
11	<.0001	<.0001	<.0001	<.0001	<.0001	0.3008	0.4520	0.1260	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
12	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.4095
13	<.0001	<.0001	<.0001	<.0001	<.0001	0.4321	0.6154	0.1995	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
14	0.5243	0.5702	0.1963	0.2028	0.9911	<.0001	<.0001	<.0001	<.0001	0.3578	0.2914	0.1790	0.9911	<.0001
15	0.1155	0.7059	0.7254	0.7395	0.3510	<.0001	<.0001	<.0001	<.0001	0.9806	0.9107	0.6866	0.3510	<.0001
16	0.0634	0.5063	0.9493	0.9642	0.2232	<.0001	<.0001	<.0001	<.0001	0.7550	0.8606	0.9078	0.2232	<.0001
17	0.5171	0.5779	0.2002	0.2068	1.0000	<.0001	<.0001	<.0001	<.0001	0.3637	0.2965	0.1826	1.0000	<.0001
18	<.0001	<.0001	<.0001	<.0001	<.0001	0.0026	0.0060	0.0005	<.0001	<.0001	<.0001	<.0001	<.0001	0.0055
19	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
20	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
21	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
22	0.9374	0.2611	0.0655	0.0683	0.5690	<.0001	<.0001	<.0001	<.0001	0.1408	0.1080	0.0584	0.5690	<.0001
23	0.0725	0.5464	0.9004	0.9152	0.2473	<.0001	<.0001	<.0001	<.0001	0.8023	0.9092	0.8591	0.2473	<.0001
24	0.0694	0.5329	0.9167	0.9315	0.2390	<.0001	<.0001	<.0001	<.0001	0.7864	0.8930	0.8753	0.2390	<.0001
25	0.5171	0.5779	0.2002	0.2068	1.0000	<.0001	<.0001	<.0001	<.0001	0.3637	0.2965	0.1826	1.0000	<.0001
26	0.0051	<.0001	<.0001	<.0001	0.0007	<.0001	<.0001	<.0001	0.2183	<.0001	<.0001	<.0001	0.0007	<.0001
27	0.0210	0.0006	<.0001	<.0001	0.0034	<.0001	<.0001	<.0001	0.0826	0.0002	<.0001	<.0001	0.0034	<.0001
28	0.0005	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	0.0007	0.6010	<.0001	<.0001	<.0001	<.0001	<.0001
29		0.2296	0.0551	0.0574	0.5171	<.0001	<.0001	<.0001	<.0001	0.1211	0.0921	0.0489	0.5171	<.0001
30	0.2296		0.4666	0.4781	0.5779	<.0001	<.0001	<.0001	<.0001	0.7240	0.6246	0.4353	0.5779	<.0001

31	0.0551	0.4666		0.9851	0.2002	<.0001	<.0001	<.0001	<.0001	0.7073	0.8110	0.9583	0.2002	<.0001
32	0.0574	0.4781	0.9851		0.2068	<.0001	<.0001	<.0001	<.0001	0.7212	0.8255	0.9434	0.2068	<.0001
33	0.5171	0.5779	0.2002	0.2068		<.0001	<.0001	<.0001	<.0001	0.3637	0.2965	0.1826	1.0000	<.0001
34	<.0001	<.0001	<.0001	<.0001	<.0001		0.7764	0.6167	0.0007	<.0001	<.0001	<.0001	<.0001	<.0001
35	<.0001	<.0001	<.0001	<.0001	<.0001	0.7764		0.4332	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001
36	<.0001	<.0001	<.0001	<.0001	<.0001	0.6167	0.4332		0.0036	<.0001	<.0001	<.0001	<.0001	<.0001
37	<.0001	<.0001	<.0001	<.0001	<.0001	0.0007	0.0003	0.0036		<.0001	<.0001	<.0001	<.0001	<.0001
38	0.1211	0.7240	0.7073	0.7212	0.3637	<.0001	<.0001	<.0001	<.0001		0.8915	0.6688	0.3637	<.0001
39	0.0921	0.6246	0.8110	0.8255	0.2965	<.0001	<.0001	<.0001	<.0001	0.8915		0.7707	0.2965	<.0001
40	0.0489	0.4353	0.9583	0.9434	0.1826	<.0001	<.0001	<.0001	<.0001	0.6688	0.7707		0.1826	<.0001
41	0.5171	0.5779	0.2002	0.2068	1.0000	<.0001	<.0001	<.0001	<.0001	0.3637	0.2965	0.1826		<.0001
42	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	
43	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0432
44	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2303
45	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.4032
46	0.5003	0.0621	0.0102	0.0107	0.1875	<.0001	<.0001	<.0001	0.0009	0.0272	0.0193	0.0088	0.1875	<.0001
47	0.0810	0.5817	0.8591	0.8738	0.2691	<.0001	<.0001	<.0001	<.0001	0.8430	0.9508	0.8182	0.2691	<.0001
48	0.0616	0.4980	0.9598	0.9747	0.2183	<.0001	<.0001	<.0001	<.0001	0.7451	0.8503	0.9181	0.2183	<.0001

i/j	43	44	45	46	47	48	49	50	51	52	53	54	55	56
1	<.0001	<.0001	<.0001	0.1875	0.2691	0.2183	1.0000	0.6312	0.4909	0.4632	0.6620	0.1025	0.7893	0.2473

2	<.0001	<.0001	<.0001	0.2155	0.0004	0.0002	0.0114	0.0028	0.0015	0.0013	0.0032	<.0001	0.0054	0.0003
3	<.0001	<.0001	<.0001	0.0082	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
4	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	<.0001	0.0740	<.0001	<.0001	0.0022	0.0005	0.0002	0.0002	0.0005	<.0001	0.0009	<.0001
6	<.0001	<.0001	<.0001	0.0227	0.9004	0.8008	0.3267	0.6154	0.7693	0.8037	0.5855	0.5099	0.4747	0.8591
7	<.0001	<.0001	<.0001	0.0120	0.9078	0.9911	0.2225	0.4576	0.5933	0.6246	0.4321	0.6756	0.3397	0.9493
8	<.0001	<.0001	<.0001	0.0124	0.9167	0.9821	0.2267	0.4644	0.6010	0.6325	0.4386	0.6675	0.3453	0.9583
9	<.0001	<.0001	<.0001	0.1875	0.2691	0.2183	1.0000	0.6312	0.4909	0.4632	0.6620	0.1025	0.7893	0.2473
10	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
11	<.0001	0.0004	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
12	0.0049	0.7059	0.9911	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
13	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
14	<.0001	<.0001	<.0001	0.1913	0.2643	0.2142	0.9911	0.6233	0.4839	0.4564	0.6539	0.1002	0.7807	0.2428
15	<.0001	<.0001	<.0001	0.0256	0.8620	0.7636	0.3510	0.6499	0.8066	0.8415	0.6193	0.4793	0.5051	0.8211
16	<.0001	<.0001	<.0001	0.0121	0.9092	0.9896	0.2232	0.4587	0.5945	0.6259	0.4332	0.6743	0.3406	0.9508
17	<.0001	<.0001	<.0001	0.1875	0.2691	0.2183	1.0000	0.6312	0.4909	0.4632	0.6620	0.1025	0.7893	0.2473
18	<.0001	0.1068	0.0487	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
20	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
21	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
22	<.0001	<.0001	<.0001	0.4520	0.0954	0.0731	0.5690	0.2948	0.2094	0.1938	0.3149	0.0286	0.4032	0.0856
23	<.0001	<.0001	<.0001	0.0143	0.9583	0.9404	0.2473	0.4968	0.6379	0.6702	0.4701	0.6299	0.3726	1.0000

24	<.0001	<.0001	<.0001	0.0135	0.9419	0.9568	0.2390	0.4839	0.6233	0.6553	0.4576	0.6445	0.3617	0.9836
25	<.0001	<.0001	<.0001	0.1875	0.2691	0.2183	1.0000	0.6312	0.4909	0.4632	0.6620	0.1025	0.7893	0.2473
26	<.0001	<.0001	<.0001	0.0315	<.0001	<.0001	0.0007	0.0001	<.0001	<.0001	0.0001	<.0001	0.0003	<.0001
27	<.0001	<.0001	<.0001	0.0987	<.0001	<.0001	0.0034	0.0007	0.0004	0.0003	0.0008	<.0001	0.0015	<.0001
28	<.0001	<.0001	<.0001	0.0046	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
29	<.0001	<.0001	<.0001	0.5003	0.0810	0.0616	0.5171	0.2604	0.1826	0.1685	0.2789	0.0235	0.3607	0.0725
30	<.0001	<.0001	<.0001	0.0621	0.5817	0.4980	0.5779	0.9389	0.8944	0.8591	0.9048	0.2789	0.7721	0.5464
31	<.0001	<.0001	<.0001	0.0102	0.8591	0.9598	0.2002	0.4212	0.5514	0.5817	0.3970	0.7212	0.3096	0.9004
32	<.0001	<.0001	<.0001	0.0107	0.8738	0.9747	0.2068	0.4321	0.5639	0.5945	0.4074	0.7073	0.3185	0.9152
33	<.0001	<.0001	<.0001	0.1875	0.2691	0.2183	1.0000	0.6312	0.4909	0.4632	0.6620	0.1025	0.7893	0.2473
34	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
35	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
36	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
37	<.0001	<.0001	<.0001	0.0009	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
38	<.0001	<.0001	<.0001	0.0272	0.8430	0.7451	0.3637	0.6675	0.8255	0.8606	0.6365	0.4644	0.5207	0.8023
39	<.0001	<.0001	<.0001	0.0193	0.9508	0.8503	0.2965	0.5715	0.7212	0.7550	0.5427	0.5514	0.4364	0.9092
40	<.0001	<.0001	<.0001	0.0088	0.8182	0.9181	0.1826	0.3918	0.5171	0.5464	0.3686	0.7607	0.2855	0.8591
41	<.0001	<.0001	<.0001	0.1875	0.2691	0.2183	1.0000	0.6312	0.4909	0.4632	0.6620	0.1025	0.7893	0.2473
42	0.0432	0.2303	0.4032	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
43		0.0015	0.0047	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
44	0.0015		0.7142	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
45	0.0047	0.7142		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

24	<.0001	<.0001	<.0001	0.0135	0.9419	0.9568	0.2390	0.4839	0.6233	0.6553	0.4576	0.6445	0.3617	0.9836
25	<.0001	<.0001	<.0001	0.1875	0.2691	0.2183	1.0000	0.6312	0.4909	0.4632	0.6620	0.1025	0.7893	0.2473
26	<.0001	<.0001	<.0001	0.0315	<.0001	<.0001	0.0007	0.0001	<.0001	<.0001	0.0001	<.0001	0.0003	<.0001
27	<.0001	<.0001	<.0001	0.0987	<.0001	<.0001	0.0034	0.0007	0.0004	0.0003	0.0008	<.0001	0.0015	<.0001
28	<.0001	<.0001	<.0001	0.0046	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
29	<.0001	<.0001	<.0001	0.5003	0.0810	0.0616	0.5171	0.2604	0.1826	0.1685	0.2789	0.0235	0.3607	0.0725
30	<.0001	<.0001	<.0001	0.0621	0.5817	0.4980	0.5779	0.9389	0.8944	0.8591	0.9048	0.2789	0.7721	0.5464
31	<.0001	<.0001	<.0001	0.0102	0.8591	0.9598	0.2002	0.4212	0.5514	0.5817	0.3970	0.7212	0.3096	0.9004
32	<.0001	<.0001	<.0001	0.0107	0.8738	0.9747	0.2068	0.4321	0.5639	0.5945	0.4074	0.7073	0.3185	0.9152
33	<.0001	<.0001	<.0001	0.1875	0.2691	0.2183	1.0000	0.6312	0.4909	0.4632	0.6620	0.1025	0.7893	0.2473
34	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
35	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
36	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
37	<.0001	<.0001	<.0001	0.0009	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
38	<.0001	<.0001	<.0001	0.0272	0.8430	0.7451	0.3637	0.6675	0.8255	0.8606	0.6365	0.4644	0.5207	0.8023
39	<.0001	<.0001	<.0001	0.0193	0.9508	0.8503	0.2965	0.5715	0.7212	0.7550	0.5427	0.5514	0.4364	0.9092
40	<.0001	<.0001	<.0001	0.0088	0.8182	0.9181	0.1826	0.3918	0.5171	0.5464	0.3686	0.7607	0.2855	0.8591
41	<.0001	<.0001	<.0001	0.1875	0.2691	0.2183	1.0000	0.6312	0.4909	0.4632	0.6620	0.1025	0.7893	0.2473
42	0.0432	0.2303	0.4032	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
43		0.0015	0.0047	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
44	0.0015		0.7142	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
45	0.0047	0.7142		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

46	<.0001	<.0001	<.0001		0.0164	0.0117	0.1875	0.0734	0.0461	0.0415	0.0804	0.0036	0.1138	0.0143
47	<.0001	<.0001	<.0001	0.0164		0.8989	0.2691	0.5304	0.6756	0.7087	0.5027	0.5933	0.4011	0.9583
48	<.0001	<.0001	<.0001	0.0117	0.8989		0.2183	0.4508	0.5855	0.6167	0.4255	0.6838	0.3340	0.9404

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14
49	1.0000	0.0114	0.0001	<.0001	0.0022	0.3267	0.2225	0.2267	1.0000	<.0001	<.0001	<.0001	<.0001	0.9911
50	0.6312	0.0028	<.0001	<.0001	0.0005	0.6154	0.4576	0.4644	0.6312	<.0001	<.0001	<.0001	<.0001	0.6233
51	0.4909	0.0015	<.0001	<.0001	0.0002	0.7693	0.5933	0.6010	0.4909	<.0001	<.0001	<.0001	<.0001	0.4839
52	0.4632	0.0013	<.0001	<.0001	0.0002	0.8037	0.6246	0.6325	0.4632	<.0001	<.0001	<.0001	<.0001	0.4564
53	0.6620	0.0032	<.0001	<.0001	0.0005	0.5855	0.4321	0.4386	0.6620	<.0001	<.0001	<.0001	<.0001	0.6539
54	0.1025	<.0001	<.0001	<.0001	<.0001	0.5099	0.6756	0.6675	0.1025	<.0001	<.0001	<.0001	<.0001	0.1002
55	0.7893	0.0054	<.0001	<.0001	0.0009	0.4747	0.3397	0.3453	0.7893	<.0001	<.0001	<.0001	<.0001	0.7807
56	0.2473	0.0003	<.0001	<.0001	<.0001	0.8591	0.9493	0.9583	0.2473	<.0001	<.0001	<.0001	<.0001	0.2428

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
49	0.3510	0.2232	1.0000	<.0001	<.0001	<.0001	<.0001	0.5690	0.2473	0.2390	1.0000	0.0007	0.0034	<.0001
50	0.6499	0.4587	0.6312	<.0001	<.0001	<.0001	<.0001	0.2948	0.4968	0.4839	0.6312	0.0001	0.0007	<.0001
51	0.8066	0.5945	0.4909	<.0001	<.0001	<.0001	<.0001	0.2094	0.6379	0.6233	0.4909	<.0001	0.0004	<.0001
52	0.8415	0.6259	0.4632	<.0001	<.0001	<.0001	<.0001	0.1938	0.6702	0.6553	0.4632	<.0001	0.0003	<.0001

53	0.6193	0.4332	0.6620	<.0001	<.0001	<.0001	<.0001	0.3149	0.4701	0.4576	0.6620	0.0001	0.0008	<.0001
54	0.4793	0.6743	0.1025	<.0001	<.0001	<.0001	<.0001	0.0286	0.6299	0.6445	0.1025	<.0001	<.0001	<.0001
55	0.5051	0.3406	0.7893	<.0001	<.0001	<.0001	<.0001	0.4032	0.3726	0.3617	0.7893	0.0003	0.0015	<.0001
56	0.8211	0.9508	0.2473	<.0001	<.0001	<.0001	<.0001	0.0856	1.0000	0.9836	0.2473	<.0001	<.0001	<.0001

i/j	29	30	31	32	33	34	35	36	37	38	39	40	41	42
49	0.5171	0.5779	0.2002	0.2068	1.0000	<.0001	<.0001	<.0001	<.0001	0.3637	0.2965	0.1826	1.0000	<.0001
50	0.2604	0.9389	0.4212	0.4321	0.6312	<.0001	<.0001	<.0001	<.0001	0.6675	0.5715	0.3918	0.6312	<.0001
51	0.1826	0.8944	0.5514	0.5639	0.4909	<.0001	<.0001	<.0001	<.0001	0.8255	0.7212	0.5171	0.4909	<.0001
52	0.1685	0.8591	0.5817	0.5945	0.4632	<.0001	<.0001	<.0001	<.0001	0.8606	0.7550	0.5464	0.4632	<.0001
53	0.2789	0.9048	0.3970	0.4074	0.6620	<.0001	<.0001	<.0001	<.0001	0.6365	0.5427	0.3686	0.6620	<.0001
54	0.0235	0.2789	0.7212	0.7073	0.1025	<.0001	<.0001	<.0001	<.0001	0.4644	0.5514	0.7607	0.1025	<.0001
55	0.3607	0.7721	0.3096	0.3185	0.7893	<.0001	<.0001	<.0001	<.0001	0.5207	0.4364	0.2855	0.7893	<.0001
56	0.0725	0.5464	0.9004	0.9152	0.2473	<.0001	<.0001	<.0001	<.0001	0.8023	0.9092	0.8591	0.2473	<.0001

i/j	43	44	45	46	47	48	49	50	51	52	53	54	55	56
49	<.0001	<.0001	<.0001	0.1875	0.2691	0.2183		0.6312	0.4909	0.4632	0.6620	0.1025	0.7893	0.2473
50	<.0001	<.0001	<.0001	0.0734	0.5304	0.4508	0.6312		0.8342	0.7994	0.9657	0.2465	0.8313	0.4968
51	<.0001	<.0001	<.0001	0.0461	0.6756	0.5855	0.4909	0.8342		0.9642	0.8008	0.3415	0.6729	0.6379
52	<.0001	<.0001	<.0001	0.0415	0.7087	0.6167	0.4632	0.7994	0.9642		0.7664	0.3646	0.6405	0.6702

53	<.0001	<.0001	<.0001	0.0804	0.5027	0.4255	0.6620	0.9657	0.8008	0.7664		0.2296	0.8650	0.4701
54	<.0001	<.0001	<.0001	0.0036	0.5933	0.6838	0.1025	0.2465	0.3415	0.3646	0.2296		0.1708	0.6299
55	<.0001	<.0001	<.0001	0.1138	0.4011	0.3340	0.7893	0.8313	0.6729	0.6405	0.8650	0.1708		0.3726
56	<.0001	<.0001	<.0001	0.0143	0.9583	0.9404	0.2473	0.4968	0.6379	0.6702	0.4701	0.6299	0.3726	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

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                    1 N inkubasie stats NO3,          10:27 Tuesday, September 2, 2003   1
                                        The GLM Procedure
                                     Class Level Information

                                      Class       Levels    Values
                                     behan          7    1 2 3 4 5 6 7
                                     per          8    1 2 3 4 5 6 7 8


                                     Number of observations    168
                    1 N inkubasie stats NO3           10:27 Tuesday, September 2, 2003   2
                                        The GLM Procedure
                                     Dependent Variable: v1   NO3

                           Sum of
          Source           DF          Squares            Mean Square        F Value       Pr > F
           Model           55          111784.6431        2032.4481          131.30        <.0001
           Error           112         1733.6963          15.4794
           Corrected Total 167         113518.3394

           R-Square        Coeff Var   Root MSE           v1           Mean
                           0.984728    11.94819           3.934391     32.92875
           Source          DF     Type I SS          Mean Square   F Value      Pr > F
           behan           6      22332.81833        3722.13639    240.46       <.0001
```

```
per                 7      61614.97104        8802.13872    568.63       <.0001
behan*per          42      27836.85374        662.78223     42.82        <.0001

 Source            DF      Type III SS        Mean Square   F Value      Pr > F
 behan              6      22332.81833        3722.13639    240.46       <.0001
per                 7      61614.97104        8802.13872    568.63       <.0001
behan*per          42      27836.85374        662.78223     42.82        <.0001

                The GLM Procedure, Least Squares Means

                     Standard           LSMEAN
      behan     v1 LSMEAN        Error   Pr > |t|   Number


      1      22.6500000    0.8031042    <.0001       1
      2      31.9475000    0.8031042    <.0001       2
      3      47.3466667    0.8031042    <.0001       3
      4      25.0416667    0.8031042    <.0001       4
      5      36.5854167    0.8031042    <.0001       5
      6      49.8950000    0.8031042    <.0001       6
      7      17.0350000    0.8031042    <.0001       7

              Least Squares Means for effect behan
             Pr > |t| for H0: LSMean(i)=LSMean(j)
                  Dependent Variable: v1
```

```
i/j      1         2         3         4         5         6         7

1                 <.0001    <.0001    0.0375    <.0001    <.0001    <.0001
2      <.0001               <.0001    <.0001    <.0001    <.0001    <.0001
3      <.0001    <.0001               <.0001    <.0001    0.0268    <.0001
4      0.0375    <.0001    <.0001               <.0001    <.0001    <.0001
5      <.0001    <.0001    <.0001    <.0001               <.0001    <.0001
6      <.0001    <.0001    0.0268    <.0001    <.0001               <.0001
7      <.0001    <.0001    <.0001    <.0001    <.0001    <.0001

                      Standard              LSMEAN
per     v1 LSMEAN        Error   Pr > |t|    Number
1       8.4566667    0.8585545    <.0001       1
2      17.6261905    0.8585545    <.0001       2
3      18.7342857    0.8585545    <.0001       3
4      21.1933333    0.8585545    <.0001       4
5      27.6266667    0.8585545    <.0001       5
6      50.7642857    0.8585545    <.0001       6
7      58.5161905    0.8585545    <.0001       7
8      60.5123810    0.8585545    <.0001       8
```

```
                    The GLM Procedure, Least Squares Means
                       Least Squares Means for effect per
                       Pr > |t| for H0: LSMean(i)=LSMean(j)
                            Dependent Variable: v1

i/j       1        2        3        4        5        6        7        8

  1             <.0001   <.0001   <.0001   <.0001   <.0001   <.0001   <.0001
  2    <.0001            0.3634   0.0040   <.0001   <.0001   <.0001   <.0001
  3    <.0001   0.3634            0.0452   <.0001   <.0001   <.0001   <.0001
  4    <.0001   0.0040   0.0452            <.0001   <.0001   <.0001   <.0001
  5    <.0001   <.0001   <.0001   <.0001            <.0001   <.0001   <.0001
  6    <.0001   <.0001   <.0001   <.0001   <.0001            <.0001   <.0001
  7    <.0001   <.0001   <.0001   <.0001   <.0001   <.0001            0.1030
  8    <.0001   <.0001   <.0001   <.0001   <.0001   <.0001   0.1030

                            Standard              LSMEAN
      behan   per    v1 LSMEAN       Error    Pr > |t|     Number
        1      1      8.456667    2.271522      0.0003          1
        1      2     12.003333    2.271522      <.0001          2
        1      3     10.376667    2.271522      <.0001          3
        1      4     11.220000    2.271522      <.0001          4
```

1	5	18.250000	2.271522	<.0001	5
1	6	32.666667	2.271522	<.0001	6
1	7	45.516667	2.271522	<.0001	7
1	8	42.710000	2.271522	<.0001	8
2	1	8.456667	2.271522	0.0003	9
2	2	9.463333	2.271522	<.0001	10
2	3	10.643333	2.271522	<.0001	11
2	4	15.756667	2.271522	<.0001	12
2	5	23.333333	2.271522	<.0001	13
2	6	55.756667	2.271522	<.0001	14
2	7	65.470000	2.271522	<.0001	15
2	8	66.700000	2.271522	<.0001	16
3	1	8.456667	2.271522	0.0003	17
3	2	9.630000	2.271522	<.0001	18
3	3	6.683333	2.271522	0.0040	19
3	4	10.413333	2.271522	<.0001	20
3	5	27.700000	2.271522	<.0001	21
3	6	92.730000	2.271522	<.0001	22
3	7	105.336667	2.271522	<.0001	23
3	8	117.823333	2.271522	<.0001	24
4	1	8.456667	2.271522	0.0003	25
4	2	17.153333	2.271522	<.0001	26
4	3	20.793333	2.271522	<.0001	27
4	4	21.876667	2.271522	<.0001	28

4	5	26.486667	2.271522	<.0001	29
4	6	36.500000	2.271522	<.0001	30
4	7	33.040000	2.271522	<.0001	31
4	8	36.026667	2.271522	<.0001	32
5	1	8.456667	2.271522	0.0003	33
5	2	26.433333	2.271522	<.0001	34
5	3	30.933333	2.271522	<.0001	35
5	4	34.793333	2.271522	<.0001	36
5	5	38.100000	2.271522	<.0001	37
5	6	50.440000	2.271522	<.0001	38
5	7	49.600000	2.271522	<.0001	39
5	8	53.926667	2.271522	<.0001	40
6	1	8.456667	2.271522	0.0003	41
6	2	38.806667	2.271522	<.0001	42
6	3	41.310000	2.271522	<.0001	43
6	4	43.400000	2.271522	<.0001	44
6	5	46.890000	2.271522	<.0001	45
6	6	70.570000	2.271522	<.0001	46
6	7	75.133333	2.271522	<.0001	47
6	8	74.593333	2.271522	<.0001	48
7	1	8.456667	2.271522	0.0003	49
7	2	9.893333	2.271522	<.0001	50
7	3	10.400000	2.271522	<.0001	51
7	4	10.893333	2.271522	<.0001	52

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              7      5       12.626667       2.271522      <.0001        53
              7      6       16.686667       2.271522      <.0001        54
              7      7       35.516667       2.271522      <.0001        55
              7      8       31.806667       2.271522      <.0001        56


                              N inkubasie stats NO3           10:27 Tuesday, September 2, 2003   6


                                        The GLM Procedure, Least Squares Means
                                        Least Squares Means for effect behan*per
                                          Pr > |t| for H0: LSMean(i)=LSMean(j)
                                               Dependent Variable: v1


i/j      1       2       3       4       5       6       7       8       9      10      11      12      13      14

  1          0.2719  0.5513  0.3915  0.0029  <.0001  <.0001  <.0001  1.0000  0.7546  0.4975  0.0250  <.0001  <.0001
  2  0.2719          0.6136  0.8078  0.0543  <.0001  <.0001  <.0001  0.2719  0.4308  0.6728  0.2451  0.0006  <.0001
  3  0.5513  0.6136          0.7934  0.0158  <.0001  <.0001  <.0001  0.5513  0.7767  0.9340  0.0968  0.0001  <.0001
  4  0.3915  0.8078  0.7934          0.0307  <.0001  <.0001  <.0001  0.3915  0.5856  0.8579  0.1607  0.0003  <.0001
  5  0.0029  0.0543  0.0158  0.0307          <.0001  <.0001  <.0001  0.0029  0.0072  0.0196  0.4393  0.1164  <.0001
  6  <.0001  <.0001  <.0001  <.0001  <.0001          0.0001  0.0023  <.0001  <.0001  <.0001  <.0001  0.0044  <.0001
  7  <.0001  <.0001  <.0001  <.0001  <.0001  0.0001          0.3842  <.0001  <.0001  <.0001  <.0001  <.0001  0.0019
  8  <.0001  <.0001  <.0001  <.0001  <.0001  0.0023  0.3842          <.0001  <.0001  <.0001  <.0001  <.0001  <.0001
  9  1.0000  0.2719  0.5513  0.3915  0.0029  <.0001  <.0001  <.0001          0.7546  0.4975  0.0250  <.0001  <.0001
 10  0.7546  0.4308  0.7767  0.5856  0.0072  <.0001  <.0001  <.0001  0.7546          0.7141  0.0526  <.0001  <.0001
```

11	0.4975	0.6728	0.9340	0.8579	0.0196	<.0001	<.0001	<.0001	0.4975	0.7141		0.1143	0.0001	<.0001
12	0.0250	0.2451	0.0968	0.1607	0.4393	<.0001	<.0001	<.0001	0.0250	0.0526	0.1143		0.0201	<.0001
13	<.0001	0.0006	0.0001	0.0003	0.1164	0.0044	<.0001	<.0001	<.0001	<.0001	0.0001	0.0201		<.0001
14	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0019	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	
15	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0031
16	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0009
17	1.0000	0.2719	0.5513	0.3915	0.0029	<.0001	<.0001	<.0001	1.0000	0.7546	0.4975	0.0250	<.0001	<.0001
18	0.7156	0.4616	0.8166	0.6216	0.0084	<.0001	<.0001	<.0001	0.7156	0.9587	0.7530	0.0591	<.0001	<.0001
19	0.5820	0.1005	0.2527	0.1607	0.0005	<.0001	<.0001	<.0001	0.5820	0.3887	0.2203	0.0056	<.0001	<.0001
20	0.5437	0.6216	0.9909	0.8022	0.0163	<.0001	<.0001	<.0001	0.5437	0.7680	0.9431	0.0990	0.0001	<.0001
21	<.0001	<.0001	<.0001	<.0001	0.0040	0.1249	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	0.1768	<.0001
22	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
23	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
24	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
25	1.0000	0.2719	0.5513	0.3915	0.0029	<.0001	<.0001	<.0001	1.0000	0.7546	0.4975	0.0250	<.0001	<.0001
26	0.0078	0.1117	0.0371	0.0674	0.7335	<.0001	<.0001	<.0001	0.0078	0.0183	0.0451	0.6646	0.0569	<.0001
27	0.0002	0.0072	0.0016	0.0035	0.4302	0.0003	<.0001	<.0001	0.0002	0.0006	0.0020	0.1197	0.4308	<.0001
28	<.0001	0.0027	0.0005	0.0012	0.2613	0.0011	<.0001	<.0001	<.0001	0.0002	0.0007	0.0593	0.6511	<.0001
29	<.0001	<.0001	<.0001	<.0001	0.0117	0.0569	<.0001	<.0001	<.0001	<.0001	<.0001	0.0011	0.3284	<.0001
30	<.0001	<.0001	<.0001	<.0001	<.0001	0.2353	0.0059	0.0557	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
31	<.0001	<.0001	<.0001	<.0001	<.0001	0.9077	0.0002	0.0032	<.0001	<.0001	<.0001	<.0001	0.0031	<.0001
32	<.0001	<.0001	<.0001	<.0001	<.0001	0.2978	0.0038	0.0398	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001
33	1.0000	0.2719	0.5513	0.3915	0.0029	<.0001	<.0001	<.0001	1.0000	0.7546	0.4975	0.0250	<.0001	<.0001
34	<.0001	<.0001	<.0001	<.0001	0.0122	0.0548	<.0001	<.0001	<.0001	<.0001	<.0001	0.0012	0.3366	<.0001

35	<.0001	<.0001	<.0001	<.0001	0.0001	0.5906	<.0001	0.0004	<.0001	<.0001	<.0001	<.0001	0.0197	<.0001
36	<.0001	<.0001	<.0001	<.0001	<.0001	0.5093	0.0011	0.0152	<.0001	<.0001	<.0001	<.0001	0.0005	<.0001
37	<.0001	<.0001	<.0001	<.0001	<.0001	0.0936	0.0228	0.1541	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
38	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1282	0.0178	<.0001	<.0001	<.0001	<.0001	<.0001	0.1007
39	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2063	0.0341	<.0001	<.0001	<.0001	<.0001	<.0001	0.0578
40	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0101	0.0007	<.0001	<.0001	<.0001	<.0001	<.0001	0.5700
41	1.0000	0.2719	0.5513	0.3915	0.0029	<.0001	<.0001	<.0001	1.0000	0.7546	0.4975	0.0250	<.0001	<.0001
42	<.0001	<.0001	<.0001	<.0001	<.0001	0.0585	0.0390	0.2269	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
43	<.0001	<.0001	<.0001	<.0001	<.0001	0.0082	0.1930	0.6638	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
44	<.0001	<.0001	<.0001	<.0001	<.0001	0.0011	0.5113	0.8303	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002
45	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.6698	0.1959	<.0001	<.0001	<.0001	<.0001	<.0001	0.0068
46	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
48	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	<.0001	<.0001	1.0000	0.7156	0.5820	0.5437	<.0001	<.0001	<.0001	<.0001	1.0000	0.0078	0.0002	<.0001
2	<.0001	<.0001	0.2719	0.4616	0.1005	0.6216	<.0001	<.0001	<.0001	<.0001	0.2719	0.1117	0.0072	0.0027
3	<.0001	<.0001	0.5513	0.8166	0.2527	0.9909	<.0001	<.0001	<.0001	<.0001	0.5513	0.0371	0.0016	0.0005
4	<.0001	<.0001	0.3915	0.6216	0.1607	0.8022	<.0001	<.0001	<.0001	<.0001	0.3915	0.0674	0.0035	0.0012
5	<.0001	<.0001	0.0029	0.0084	0.0005	0.0163	0.0040	<.0001	<.0001	<.0001	0.0029	0.7335	0.4302	0.2613
6	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1249	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	0.0011
7	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

8	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
9	<.0001	<.0001	1.0000	0.7156	0.5820	0.5437	<.0001	<.0001	<.0001	<.0001	1.0000	0.0078	0.0002	<.0001
10	<.0001	<.0001	0.7546	0.9587	0.3887	0.7680	<.0001	<.0001	<.0001	<.0001	0.7546	0.0183	0.0006	0.0002
11	<.0001	<.0001	0.4975	0.7530	0.2203	0.9431	<.0001	<.0001	<.0001	<.0001	0.4975	0.0451	0.0020	0.0007
12	<.0001	<.0001	0.0250	0.0591	0.0056	0.0990	0.0003	<.0001	<.0001	<.0001	0.0250	0.6646	0.1197	0.0593
13	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.1768	<.0001	<.0001	<.0001	<.0001	0.0569	0.4308	0.6511
14	0.0031	0.0009	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
15		0.7025	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
16	0.7025		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
17	<.0001	<.0001		0.7156	0.5820	0.5437	<.0001	<.0001	<.0001	<.0001	1.0000	0.0078	0.0002	<.0001
18	<.0001	<.0001	0.7156		0.3610	0.8078	<.0001	<.0001	<.0001	<.0001	0.7156	0.0210	0.0007	0.0002
19	<.0001	<.0001	0.5820	0.3610		0.2481	<.0001	<.0001	<.0001	<.0001	0.5820	0.0015	<.0001	<.0001
20	<.0001	<.0001	0.5437	0.8078	0.2481		<.0001	<.0001	<.0001	<.0001	0.5437	0.0381	0.0016	0.0005
21	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	0.0014	0.0337	0.0725
22	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.0002	<.0001	<.0001	<.0001	<.0001	<.0001
23	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002		0.0002	<.0001	<.0001	<.0001	<.0001
24	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002		<.0001	<.0001	<.0001	<.0001
25	<.0001	<.0001	1.0000	0.7156	0.5820	0.5437	<.0001	<.0001	<.0001	<.0001		0.0078	0.0002	<.0001
26	<.0001	<.0001	0.0078	0.0210	0.0015	0.0381	0.0014	<.0001	<.0001	<.0001	0.0078		0.2596	0.1443
27	<.0001	<.0001	0.0002	0.0007	<.0001	0.0016	0.0337	<.0001	<.0001	<.0001	0.0002	0.2596		0.7366
28	<.0001	<.0001	<.0001	0.0002	<.0001	0.0005	0.0725	<.0001	<.0001	<.0001	<.0001	0.1443	0.7366	
29	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.7064	<.0001	<.0001	<.0001	<.0001	0.0044	0.0791	0.1541
30	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0072	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
31	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0992	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	0.0007

32	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0108	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
33	<.0001	<.0001	1.0000	0.7156	0.5820	0.5437	<.0001	<.0001	<.0001	<.0001	1.0000	0.0078	0.0002	<.0001
34	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.6941	<.0001	<.0001	<.0001	<.0001	0.0046	0.0819	0.1588
35	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.3163	<.0001	<.0001	<.0001	<.0001	<.0001	0.0021	0.0057
36	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0293	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001
37	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0016	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
38	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
39	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
40	0.0005	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
41	<.0001	<.0001	1.0000	0.7156	0.5820	0.5437	<.0001	<.0001	<.0001	<.0001	1.0000	0.0078	0.0002	<.0001
42	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0008	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
43	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
44	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
45	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
46	0.1152	0.2309	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	0.0032	0.0099	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
48	0.0054	0.0155	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

i/j	29	30	31	32	33	34	35	36	37	38	39	40	41	42
1	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001
2	<.0001	<.0001	<.0001	<.0001	0.2719	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2719	<.0001
3	<.0001	<.0001	<.0001	<.0001	0.5513	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5513	<.0001
4	<.0001	<.0001	<.0001	<.0001	0.3915	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.3915	<.0001

5	0.0117	<.0001	<.0001	<.0001	0.0029	0.0122	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0029	<.0001
6	0.0569	0.2353	0.9077	0.2978	<.0001	0.0548	0.5906	0.5093	0.0936	<.0001	<.0001	<.0001	<.0001	0.0585
7	<.0001	0.0059	0.0002	0.0038	<.0001	<.0001	<.0001	0.0011	0.0228	0.1282	0.2063	0.0101	<.0001	0.0390
8	<.0001	0.0557	0.0032	0.0398	<.0001	<.0001	0.0004	0.0152	0.1541	0.0178	0.0341	0.0007	<.0001	0.2269
9	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001
10	<.0001	<.0001	<.0001	<.0001	0.7546	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.7546	<.0001
11	<.0001	<.0001	<.0001	<.0001	0.4975	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.4975	<.0001
12	0.0011	<.0001	<.0001	<.0001	0.0250	0.0012	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0250	<.0001
13	0.3284	<.0001	0.0031	0.0001	<.0001	0.3366	0.0197	0.0005	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
14	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1007	0.0578	0.5700	<.0001	<.0001
15	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0005	<.0001	<.0001
16	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001
17	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001
18	<.0001	<.0001	<.0001	<.0001	0.7156	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.7156	<.0001
19	<.0001	<.0001	<.0001	<.0001	0.5820	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5820	<.0001
20	<.0001	<.0001	<.0001	<.0001	0.5437	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5437	<.0001
21	0.7064	0.0072	0.0992	0.0108	<.0001	0.6941	0.3163	0.0293	0.0016	<.0001	<.0001	<.0001	<.0001	0.0008
22	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
23	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
24	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
25	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001
26	0.0044	<.0001	<.0001	<.0001	0.0078	0.0046	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0078	<.0001
27	0.0791	<.0001	0.0002	<.0001	0.0002	0.0819	0.0021	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	<.0001
28	0.1541	<.0001	0.0007	<.0001	<.0001	0.1588	0.0057	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

29		0.0023	0.0437	0.0036	<.0001	0.9868	0.1690	0.0110	0.0005	<.0001	<.0001	<.0001	<.0001	0.0002
30	0.0023		0.2838	0.8831	<.0001	0.0022	0.0859	0.5963	0.6194	<.0001	<.0001	<.0001	<.0001	0.4742
31	0.0437	0.2838		0.3545	<.0001	0.0420	0.5133	0.5863	0.1180	<.0001	<.0001	<.0001	<.0001	0.0753
32	0.0036	0.8831	0.3545		<.0001	0.0035	0.1157	0.7018	0.5200	<.0001	<.0001	<.0001	<.0001	0.3887
33	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001
34	0.9868	0.0022	0.0420	0.0035	<.0001		0.1640	0.0105	0.0004	<.0001	<.0001	<.0001	<.0001	0.0002
35	0.1690	0.0859	0.5133	0.1157	<.0001	0.1640		0.2321	0.0277	<.0001	<.0001	<.0001	<.0001	0.0158
36	0.0110	0.5963	0.5863	0.7018	<.0001	0.0105	0.2321		0.3055	<.0001	<.0001	<.0001	<.0001	0.2142
37	0.0005	0.6194	0.1180	0.5200	<.0001	0.0004	0.0277	0.3055		0.0002	0.0005	<.0001	<.0001	0.8263
38	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002		0.7942	0.2801	<.0001	0.0004
39	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0005	0.7942		0.1807	<.0001	0.0011
40	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2801	0.1807		<.0001	<.0001
41	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
42	0.0002	0.4742	0.0753	0.3887	<.0001	0.0002	0.0158	0.2142	0.8263	0.0004	0.0011	<.0001	<.0001	
43	<.0001	0.1371	0.0113	0.1028	<.0001	<.0001	0.0016	0.0449	0.3198	0.0053	0.0112	0.0001	<.0001	0.4375
44	<.0001	0.0339	0.0017	0.0236	<.0001	<.0001	0.0002	0.0085	0.1018	0.0305	0.0561	0.0014	<.0001	0.1555
45	<.0001	0.0016	<.0001	0.0010	<.0001	<.0001	<.0001	0.0003	0.0072	0.2715	0.4007	0.0306	<.0001	0.0133
46	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
48	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

i/j	43	44	45	46	47	48	49	50	51	52	53	54	55	56
1	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	0.6556	0.5464	0.4497	0.1969	0.0117	<.0001	<.0001

2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2719	0.5126	0.6187	0.7303	0.8465	0.1477	<.0001	<.0001
3	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5513	0.8807	0.9942	0.8725	0.4851	0.0520	<.0001	<.0001
4	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.3915	0.6804	0.7990	0.9192	0.6623	0.0916	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0029	0.0105	0.0161	0.0239	0.0828	0.6275	<.0001	<.0001
6	0.0082	0.0011	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.3769	0.7894
7	0.1930	0.5113	0.6698	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0024	<.0001
8	0.6638	0.8303	0.1959	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0271	0.0010
9	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	0.6556	0.5464	0.4497	0.1969	0.0117	<.0001	<.0001
10	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.7546	0.8938	0.7711	0.6571	0.3269	0.0265	<.0001	<.0001
11	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.4975	0.8158	0.9398	0.9381	0.5382	0.0625	<.0001	<.0001
12	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0250	0.0706	0.0982	0.1329	0.3320	0.7727	<.0001	<.0001
13	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.0002	0.0012	0.0408	0.0002	0.0095
14	<.0001	0.0002	0.0068	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
15	<.0001	<.0001	<.0001	0.1152	0.0032	0.0054	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
16	<.0001	<.0001	<.0001	0.2309	0.0099	0.0155	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
17	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	0.6556	0.5464	0.4497	0.1969	0.0117	<.0001	<.0001
18	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.7156	0.9348	0.8110	0.6949	0.3529	0.0301	<.0001	<.0001
19	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5820	0.3198	0.2497	0.1927	0.0669	0.0023	<.0001	<.0001
20	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5437	0.8717	0.9967	0.8815	0.4923	0.0533	<.0001	<.0001
21	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0009	0.0165	0.2038
22	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
23	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
24	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
25	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	0.6556	0.5464	0.4497	0.1969	0.0117	<.0001	<.0001

26	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0078	0.0258	0.0378	0.0538	0.1616	0.8848	<.0001	<.0001
27	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002	0.0010	0.0016	0.0026	0.0124	0.2038	<.0001	0.0009
28	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	0.0005	0.0009	0.0048	0.1090	<.0001	0.0025
29	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0029	0.0058	0.1005
30	0.1371	0.0339	0.0016	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.7601	0.1468
31	0.0113	0.0017	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.4424	0.7018
32	0.1028	0.0236	0.0010	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.8741	0.1916
33	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	0.6556	0.5464	0.4497	0.1969	0.0117	<.0001	<.0001
34	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0030	0.0056	0.0972
35	0.0016	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1564	0.7862
36	0.0449	0.0085	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.8223	0.3545
37	0.3198	0.1018	0.0072	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.4230	0.0526
38	0.0053	0.0305	0.2715	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
39	0.0112	0.0561	0.4007	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
40	0.0001	0.0014	0.0306	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
41	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	0.6556	0.5464	0.4497	0.1969	0.0117	<.0001	<.0001
42	0.4375	0.1555	0.0133	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.3080	0.0314
43		0.5166	0.0851	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0740	0.0038
44	0.5166		0.2796	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0157	0.0005
45	0.0851	0.2796		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0006	<.0001
46	<.0001	<.0001	<.0001		0.1582	0.2130	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
47	<.0001	<.0001	<.0001	0.1582		0.8668	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
48	<.0001	<.0001	<.0001	0.2130	0.8668		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13	14
49	1.0000	0.2719	0.5513	0.3915	0.0029	<.0001	<.0001	<.0001	1.0000	0.7546	0.4975	0.0250	<.0001	<.0001
50	0.6556	0.5126	0.8807	0.6804	0.0105	<.0001	<.0001	<.0001	0.6556	0.8938	0.8158	0.0706	<.0001	<.0001
51	0.5464	0.6187	0.9942	0.7990	0.0161	<.0001	<.0001	<.0001	0.5464	0.7711	0.9398	0.0982	0.0001	<.0001
52	0.4497	0.7303	0.8725	0.9192	0.0239	<.0001	<.0001	<.0001	0.4497	0.6571	0.9381	0.1329	0.0002	<.0001
53	0.1969	0.8465	0.4851	0.6623	0.0828	<.0001	<.0001	<.0001	0.1969	0.3269	0.5382	0.3320	0.0012	<.0001
54	0.0117	0.1477	0.0520	0.0916	0.6275	<.0001	<.0001	<.0001	0.0117	0.0265	0.0625	0.7727	0.0408	<.0001
55	<.0001	<.0001	<.0001	<.0001	<.0001	0.3769	0.0024	0.0271	<.0001	<.0001	<.0001	<.0001	0.0002	<.0001
56	<.0001	<.0001	<.0001	<.0001	<.0001	0.7894	<.0001	0.0010	<.0001	<.0001	<.0001	<.0001	0.0095	<.0001

i/j	15	16	17	18	19	20	21	22	23	24	25	26	27	28
49	<.0001	<.0001	1.0000	0.7156	0.5820	0.5437	<.0001	<.0001	<.0001	<.0001	1.0000	0.0078	0.0002	<.0001
50	<.0001	<.0001	0.6556	0.9348	0.3198	0.8717	<.0001	<.0001	<.0001	<.0001	0.6556	0.0258	0.0010	0.0003
51	<.0001	<.0001	0.5464	0.8110	0.2497	0.9967	<.0001	<.0001	<.0001	<.0001	0.5464	0.0378	0.0016	0.0005
52	<.0001	<.0001	0.4497	0.6949	0.1927	0.8815	<.0001	<.0001	<.0001	<.0001	0.4497	0.0538	0.0026	0.0009
53	<.0001	<.0001	0.1969	0.3529	0.0669	0.4923	<.0001	<.0001	<.0001	<.0001	0.1969	0.1616	0.0124	0.0048
54	<.0001	<.0001	0.0117	0.0301	0.0023	0.0533	0.0009	<.0001	<.0001	<.0001	0.0117	0.8848	0.2038	0.1090
55	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0165	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
56	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2038	<.0001	<.0001	<.0001	<.0001	<.0001	0.0009	0.0025

i/j	29	30	31	32	33	34	35	36	37	38	39	40	41	42
49	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	<.0001
50	<.0001	<.0001	<.0001	<.0001	0.6556	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.6556	<.0001
51	<.0001	<.0001	<.0001	<.0001	0.5464	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5464	<.0001
52	<.0001	<.0001	<.0001	<.0001	0.4497	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.4497	<.0001
53	<.0001	<.0001	<.0001	<.0001	0.1969	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1969	<.0001
54	0.0029	<.0001	<.0001	<.0001	0.0117	0.0030	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0117	<.0001
55	0.0058	0.7601	0.4424	0.8741	<.0001	0.0056	0.1564	0.8223	0.4230	<.0001	<.0001	<.0001	<.0001	0.3080
56	0.1005	0.1468	0.7018	0.1916	<.0001	0.0972	0.7862	0.3545	0.0526	<.0001	<.0001	<.0001	<.0001	0.0314

i/j	43	44	45	46	47	48	49	50	51	52	53	54	55	56
49	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.6556	0.5464	0.4497	0.1969	0.0117	<.0001	<.0001
50	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.6556		0.8750	0.7562	0.3967	0.0367	<.0001	<.0001
51	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5464	0.8750		0.8782	0.4897	0.0528	<.0001	<.0001
52	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.4497	0.7562	0.8782		0.5906	0.0740	<.0001	<.0001
53	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.1969	0.3967	0.4897	0.5906		0.2089	<.0001	<.0001
54	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0117	0.0367	0.0528	0.0740	0.2089		<.0001	<.0001
55	0.0740	0.0157	0.0006	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.2506
56	0.0038	0.0005	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2506	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

APPENDIX 2:

STATISTICAL ANALYSIS OF SUMMER TRIAL

Summer trial

Abbreviations

Beh: Behandeling (Treatment)
Per: Periode (Period; Time intervals)
Her: Herhaling (Repetition)

Statistical numbers		Corresponding experimental treatments
Beh	1	Control
	2	Fertilizer
	3	20 ton/ha
	4	20 ton/ha + crops

Statistical numbers		Corresponding experimental days
Per	1	0
	2	18-Oct
	3	16-Nov
	4	02-Nov
	5	03-Dec
	6	19-Dec
	7	11-Jan

```
0-30NH4                 08:33 Monday,           October 7, 2002   1
                                  The GLM Procedure
                                Class Level Information
                              Class       Levels    Values
                              beh            4    1 2 3 4
                              per            7    1 2 3 4 5 6 7
                              her            2    1 2
                                Number of observations    56


                                         Sum of
            Source                 DF        Squares    Mean Square   F Value    Pr > F
            Model                  27    18702.82724      692.69731      3.10    0.0020
            Error                  28     6257.82470      223.49374
            Corrected Total        55    24960.65194


                         R-Square    Coeff Var     Root MSE     V1 Mean
                         0.749292     75.16597     14.94971    19.88893


            Source                 DF      Type I SS    Mean Square   F Value    Pr > F
            beh                     3    8625.288636    2875.096212     12.86    <.0001
            per                     6    6428.580361    1071.430060      4.79    0.0018
            beh*per                18    3648.958239     202.719902      0.91    0.5768

            Source                 DF    Type III SS    Mean Square   F Value    Pr > F
            beh                     3    8625.288636    2875.096212     12.86    <.0001
            per                     6    6428.580361    1071.430060      4.79    0.0018
            beh*per                18    3648.958239     202.719902      0.91    0.5768

                         Alpha                                0.05
                         Error Degrees of Freedom                  28
                         Error Mean Square                   223.4937
                         Critical Value of Studentized Range  3.86125
                         Minimum Significant Difference        15.428

                       Tukey Grouping        Mean      N    beh
                               A        31.510     14    2
                               A        30.253     14    3
                               A        17.019     14    4
                               B         0.774     14    1
                         Alpha                                0.05
                         Error Degrees of Freedom                  28
                         Error Mean Square                   223.4937
                         Critical Value of Studentized Range  4.48607
                         Minimum Significant Difference        23.711
```

```
Tukey Grouping          Mean        N    per
          A            34.782       8      2
          A            30.135       8      4
          A            24.971       8      3
          B   A        21.220       8      5
          B   A        14.976       8      6
          B   A        12.493       8      7
          B             0.645       8      1

    Level of   Level of         ----------V1---------
    beh          per      N        Mean        Std Dev

    1            1        2     0.7450000    0.4171930
    1            2        2     1.9750000    2.1142493
    1            3        2     0.6000000    0.0989949
    1            4        2     0.4050000    0.3323402
    1            5        2     0.4750000    0.0777817
    1            6        2     0.9500000    0.4384062
    1            7        2     0.2650000    0.1767767
    2            1        2     0.7450000    0.4171930
    2            2        2    40.6600000   17.4231111
    2            3        2    50.6650000   28.8428856
    2            4        2    46.0450000   20.9657161
    2            5        2    36.5100000   15.6412020
    2            6        2    30.1650000   20.5273099
    2            7        2    15.7800000    4.6951890
    3            1        2     0.5450000    0.0212132
    3            2        2    54.3900000   11.5824091
    3            3        2    25.0750000   24.8123770
    3            4        2    46.8300000   37.9150656
    3            5        2    31.8350000    8.9449008
    3            6        2    26.2150000   21.4324065
    3            7        2    26.8800000   13.3218918
    4            1        2     0.5450000    0.0212132
    4            2        2    42.1050000   17.3736136
    4            3        2    23.5450000   17.1473394
    4            4        2    27.2600000    3.4223968
    4            5        2    16.0600000   21.0859242
    4            6        2     2.5750000    2.5385133
    4            7        2     7.0450000    5.1689506
```

```
030NO3                   11:18 Monday,          October 7, 2002   1

                             The GLM Procedure
                          Class Level Information
                         Class       Levels   Values
                          beh           4    1 2 3 4
                        per           7    1 2 3 4 5 6 7
                              her           2    1 2

                        Number of observations    56

                             The GLM Procedure

                        Dependent Variable: V2   no3

                        Sum of
Source                  DF       Squares            Mean Square   F Value        Pr > F
 Model                  27        5888.237436       218.082868     2.31          0.0157
 Error                  28        2643.225700        94.400918
 Corrected Total        55        8531.463136

            R-Square     Coeff Var     Root MSE      V2 Mean
             0.690179     85.22018      9.716013     11.40107

Source                DF      Type I SS      Mean Square    F Value    Pr > F
 beh                   3    2384.021864     794.673955      8.42    0.0004
per                   6    1329.909886    221.651648      2.35    0.0581
beh*per                18     2174.305686     120.794760      1.28    0.2722

 Source                DF    Type III SS     Mean Square    F Value    Pr > F
 beh                   3    2384.021864     794.673955      8.42    0.0004
per                   6    1329.909886    221.651648      2.35    0.0581
beh*per                18     2174.305686     120.794760      1.28    0.2722

              Alpha                          0.05
              Error Degrees of Freedom              28
              Error Mean Square               94.40092
              Critical Value of Studentized Range  3.86125
              Minimum Significant Difference        10.027

           Means with the same letter are not significantly different

             Tukey Grouping          Mean               N            beh
                     A               21.483             14           2
                     B               11.064             14           4
                     B               9.774              14           3
                     B               3.284              14           1

              Alpha                          0.05
              Error Degrees of Freedom              28
              Error Mean Square               94.40092
              Critical Value of Studentized Range  4.48607
              Minimum Significant Difference        15.41


             Tukey Grouping           Mean               N            per

                      A               18.406             8            2
                 B    A               16.191             8            3
                 B    A               13.304             8            4
                 B    A               11.850             8            7
                 B    A               8.921              8            5
                 B    A                8.413             8            6
                 B                    2.722              8            1
```

Level of beh	Level of per	N	V2 Mean	V2 Std Dev
1	1	2	3.0100000	0.3394113
1	2	2	4.1700000	0.3535534
1	3	2	5.1250000	0.3181981
1	4	2	3.8500000	1.0040916
1	5	2	2.5150000	0.8273149
1	6	2	2.4200000	0.7353911
1	7	2	1.9000000	0.7919596
2	1	2	3.0100000	0.3394113
2	2	2	42.4600000	33.7997041
2	3	2	31.5950000	3.3446151
2	4	2	30.0800000	11.0732922
2	5	2	20.6250000	0.7990307
2	6	2	13.3150000	12.3672976
2	7	2	9.2950000	1.5485639
3	1	2	2.4350000	0.2333452
3	2	2	11.7900000	5.3457273
3	3	2	12.0150000	9.7792868
3	4	2	8.7750000	2.9910617
3	5	2	9.0450000	1.3505740
3	6	2	14.8200000	3.5355339
3	7	2	9.5350000	1.2515790
4	1	2	2.4350000	0.2333452
4	2	2	15.2050000	11.8440386
4	3	2	16.0300000	3.5779603
4	4	2	10.5100000	4.6527626
4	5	2	3.5000000	2.5738687
4	6	2	3.0950000	1.8879751
4	7	2	26.6700000	29.5570635

Summer trial: 30-60 cm depth, NH_4 levels

```
                         The GLM Procedure
                     Class Level Information
                     Class       Levels    Values
                      beh           4     1 2 3 4
                     per          7    1 2 3 4 5 6 7
                        her           2    1 2
                     Number of observations    56

                30-6NH4                  11:18 Monday,
                          October 7, 2002   2

                    The GLM Procedure
Dependent Variable: V1   nh4

                              Sum of
    Source               DF      Squares       Mean Square   F Value       Pr > F
     Model               27    1150 866093     42.624670     1.15          0.3543
     Error               28    1034.500300     36.946439
     Corrected Total     55    2185 366393

              R-Square    Coeff Var    Root MSE     V1 Mean
              0.526624    221.4050     6.078358     2.745357


    Source               DF     Type I SS    Mean Square   F Value   Pr > F
     beh                  3   372.4963500    124.1654500     3.36    0.0327
     per                 6   205.2518929    34.2086488     0.93    0.4916
     beh*per              18   573.1178500     31.8398806     0.86    0.6222

     Source               DF    Type III SS   Mean Square    F Value   Pr > F
     beh                  3   372.4963500    124.1654500     3.36    0.0327
     per                 6   205.2518929    34.2086488     0.93    0.4916
     beh*per              18   573.1178500     31.8398806     0.86    0.6222
1                          30-6NH4                    11:18 Monday, October 7, 2002   3

               Alpha                          0.05
               Error Degrees of Freedom             28
               Error Mean Square                36.94644
               Critical Value of Studentized Range  3.86125
               Minimum Significant Difference       6.2726

             Means with the same letter are not significantly different.

              Tukey Grouping        Mean     N    beh
                     A        7.043     14   2
                 B   A        2.297     14   3
                 B   A        1.335     14   4
                 B           0.306     14   1

               Alpha                          0.05
               Error Degrees of Freedom             28
               Error Mean Square                36.94644
               Critical Value of Studentized Range  4.48607
               Minimum Significant Difference       9.6407

             Means with the same letter are not significantly different.

             Tukey Grouping          Mean      N          per
                   A                 5.934     8          6
                   A                 5.337     8          5
                   A                 2.161     8          4
                   A                 2.082     8          2
                   A                 1.676     8          7
                   A                 1.664     8          3
                   A                 0.362     8          1
```

```
1                           30-6NH4                  11 18 Monday, October 7, 2002   5

                          The GLM Procedure

  Level of    Level of          --------------V1-------------
  beh          per        N         Mean          Std Dev

  1            1          2      0.4900000      0.0989949
  1            2          2      0.0400000      0.0565685
  1            3          2      0.3400000      0.0000000
  1            4          2      0.6400000      0.1979899
  1            5          2      0.2400000      0.3394113
  1            6          2      0.2400000      0.1414214
  1            7          2      0.1550000      0.1767767
  2            1          2      0.4900000      0.0989949
  2            2          2      2.6200000      2.1213203
  2            3          2      3.6950000      2.4960869
  2            4          2      3.7250000      1.2657211
  2            5          2     18.9300000     22.0193052
  2            6          2     17.8800000     21.8354574
  2            7          2      1.9600000      0.1979899
  3            1          2      0.2350000      0.2192031
  3            2          2      2.2400000      1.1879394
  3            3          2      1.8350000      0.2616295
  3            4          2      2.7400000      2.7718586
  3            5          2      1.2300000      1.1879394
  3            6          2      4.9150000      6.3993164
  3            7          2      2.8850000      1.6192745
  4            1          2      0.2350000      0.2192031
  4            2          2      3.4300000      1.6829141
  4            3          2      0.7850000      0.3606245
  4            4          2      1.5400000      0.3111270
  4            5          2      0.9500000      0.9475231
  4            6          2      0.7000000      0.6788225
  4            7          2      1.7050000      1.2657211
```

Summer trial, 30-60 cm depth, NO_3 levels

```
                      The GLM Procedure
                   Class Level Information
                  Class      Levels   Values
                   beh          4    1 2 3 4
                  per         7   1 2 3 4 5 6 7
                      her          2    1 2

                  Number of observations    56
                  Dependent Variable: V2   no3

                             Sum of
Source                  DF         Squares         Mean Square    F Value       Pr > F
 Model                  27         8436.129205     312.449230      6.59         <.0001
 Error                  28         1328.459750      47.444991
 Corrected Total        55         9764.588955

            R-Square    Coeff Var     Root MSE      V2 Mean
            0.863951     62.58495     6.888032     11.00589

 Source                 DF     Type I SS      Mean Square     F Value    Pr > F
 beh                    3    3998.284705     1332.761568      28.09      <.0001
 per                    6    1668.841043      278.140174       5.86      0.0005
 beh*per                18    2769.003457     153.833525       3.24      0.0026


 Source                 DF    Type III SS     Mean Square    F Value    Pr > F

 beh                    3    3998.284705    1332.761568    28.09   <.0001
 per                    6    1668.841043     278.140174     5.86   0.0005
 beh*per                18    2769.003457     153.833525    3.24   0.0026

              Alpha                               0.05
              Error Degrees of Freedom                   28
              Error Mean Square                     47.44499
              Critical Value of Studentized Range  3.86125
              Minimum Significant Difference        7.1082

          Means with the same letter are not significantly different.

          Tukey Grouping          Mean      N          beh

                 A                25.356    14         2
                 B                7.709     14         3
                 B                7.440     14         4
                 B                3.518     14         1

              Alpha                               0.05
              Error Degrees of Freedom                   28
              Error Mean Square                     47.44499
              Critical Value of Studentized Range  4.48607
              Minimum Significant Difference        10.925

              Tukey Grouping          Mean     N   per

                   A                  18.121    8   5
                B   A                 16.726    8   4
                B   A   C             13.503    8   6
                B   A   C             13.360    8   7
                B       C             7.090    8   3
                        C             5.411    8   2
                      C       2.830     8   1
```

```
Level of   Level of          --------------V2-------------
beh          per       N          Mean            Std Dev

1            1         2     2.6350000       0.0777817
1            2         2     4.3250000       0.7283200
1            3         2     4.0000000       0.7919596
1            4         2     6.7750000       1.8172644
1            5         2     3.0700000       1.6122035
1            6         2     1.7200000       0.4525483
1            7         2     2.1000000       0.1131371
2            1         2     2.6350000       0.0777817
2            2         2     5.2350000       0.9121677
2            3         2    14.0850000      13.3431050
2            4         2    36.1200000      20.7465130
2            5         2    49.9400000      17.5221060
2            6         2    40.1100000       1.3293607
2            7         2    29.3700000       7.8771695
3            1         2     3.0250000       0.6717514
3            2         2     4.4950000       1.0535891
3            3         2     3.7500000       0.3959798
3            4         2     9.4100000       6.9720729
3            5         2    12.8950000      13.0036937
3            6         2     9.4800000       3.9032294
3            7         2    10.9100000       1.6829141
4            1         2     3.0250000       0.6717514
4            2         2     7.5900000       1.9798990
4            3         2     6.5250000       1.3505740
4            4         2    14.6000000       7.8630274
4            5         2     6.5800000       5.2608745
4            6         2     2.7000000       2.5173001
4            7         2    11.0600000       1.2303658
```

Summer trial, 60-90 cm depth, NH_4 levels

```
                          The GLM Procedure
                       Class Level Information
                        Class      Levels   Values
                         beh          4    1 2 3 4
                       per          7   1 2 3 4 5 6 7
                           her          2    1 2

                       Number of observations   56
Dependent Variable: V1   nh4

                              Sum of
     Source                DF      Squares          Mean Square   F Value     Pr > F
      Model                27      76.9614482        2.8504240    1.88         0.0516
      Error                28      42.5009500        1.5178911
      Corrected Total      55      119.4623982

            R-Square    Coeff Var    Root MSE     V1 Mean
            0.644232    121.3606     1.232027    1.015179

     Source                DF     Type I SS     Mean Square   F Value   Pr > F
      beh                  3    18.75789107    6.25263036     4.12    0.0154
     per                   6    18.74348571    3.12391429     2.06    0.0908
     beh*per               18    39.46007143    2.19222619     1.44    0.1864


     Source                DF    Type III SS    Mean Square    F Value   Pr > F

     beh                   3    18.75789107    6.25263036     4.12    0.0154
     per                    6    18.74348571    3.12391429     2.06    0.0908
     beh*per                18    39.46007143    2.19222619     1.44    0.1864

                 Alpha                              0.05
                 Error Degrees of Freedom                28
                 Error Mean Square                 1.517891
                 Critical Value of Studentized Range  3.86125
                 Minimum Significant Difference      1.2714

               Means with the same letter are not significantly different.

                 Tukey Grouping        Mean      N    beh

                        A       1.8721     14   2
                     B    A       1.1436     14    3
                     B    A       0.7500     14    4
                     B          0.2950     14    1

                 Alpha                              0.05
                 Error Degrees of Freedom                28
                 Error Mean Square                 1.517891
                 Critical Value of Studentized Range  4.48607
                 Minimum Significant Difference      1.9541


               Means with the same letter are not significantly different.


                 Tukey Grouping        Mean        N          per

                        A              2.1912      8          2
                     B    A            1.2512      8          4
                     B    A            1.1950      8          7
                     B    A            0.8763      8          5
                     B    A             0.7400      8          3
                     B    A            0.6300      8           6
                     B                 0.2225      8          1
```

```
1                              60-9NH4                  11:18 Monday, October 7, 2002   5

                            The GLM Procedure

              Level of    Level of              --------------V1-------------
              beh         per        N          Mean             Std Dev

              1           1          2    0.18000000      0.25455844
              1           2          2    0.18000000      0.25455844
              1           3          2    0.17000000      0.24041631
              1           4          2    0.89500000      1.26572114
              1           5          2    0.28000000      0.04242641
              1           6          2    0.24500000      0.34648232
              1           7          2    0.11500000      0.12020815
              2           1          2    0.18000000      0.25455844
              2           2          2    6.28500000      5.21137698
              2           3          2    0.59000000      0.39597980
              2           4          2    1.82000000      0.55154329
              2           5          2    1.53000000      1.48492424
              2           6          2    1.02000000      0.36769553
              2           7          2    1.68000000      0.08485281
              3           1          2    0.26500000      0.02121320
              3           2          2    1.46000000      0.39597980
              3           3          2    0.68500000      0.02121320
              3           4          2    2.02000000      0.24041631
              3           5          2    1.01000000      1.14551299
              3           6          2    0.98000000      0.83438600
              3           7          2    1.58500000      1.80312229
              4           1          2    0.26500000      0.02121320
              4           2          2    0.84000000      0.87681241
              4           3          2    1.51500000      1.66170094
              4           4          2    0.27000000      0.12727922
              4           5          2    0.68500000      0.33234019
              4           6          2    0.27500000      0.00707107
              4           7          2    1.40000000      1.18793939
```

Summer trial, 60-90 cm depth, NO_3 levels

```
                              The GLM Procedure
                            Class Level Information
                           Class       Levels    Values
                            beh            4    1 2 3 4
                          per            7    1 2 3 4 5 6 7
                              her            2    1 2

                           Number of observations    56
Dependent Variable: V2   no3

                           Sum of
            Source                DF         Squares            Mean Square   F Value    Pr > F
             Model                27         710.018734         26.296990     1.84          0.0573
             Error                 28        400.110850         14.289673
             Corrected Total      55         1110.129584

                  R-Square     Coeff Var     Root MSE      V2 Mean
                  0.639582     63.18144      3.780168     5.983036

            Source              DF      Type I SS      Mean Square    F Value    Pr > F

            beh                3     241.4215768      80.4738589      5.63     0.0038
            per               6     196.3558214     32.7259702      2.29    0.0635
            beh*per             18     272.2413357     15.1245187      1.06    0.4353

             Source              DF     Type III SS     Mean Square    F Value    Pr > F

            beh                3     241.4215768      80.4738589      5.63    0.0038
            per               6     196.3558214     32.7259702      2.29    0.0635
            beh*per             18     272.2413357     15.1245187      1.06    0.4353
1                                 6090NO3                         11:18 Monday, October 7, 2002   3

                 Alpha                          0.05
                 Error Degrees of Freedom                  28
                 Error Mean Square                  14.28967
                 Critical Value of Studentized Range  3.86125
                 Minimum Significant Difference         3.901

               Means with the same letter are not significantly different.

                Tukey Grouping          Mean        N           beh

                      A                  9.539     14           2
                      B                  5.290     14            4
                      B                  4.664     14            3
                      B                  4.439     14            1

                 Alpha                          0.05
                 Error Degrees of Freedom                  28
                 Error Mean Square                  14.28967
                 Critical Value of Studentized Range  4.48607
                 Minimum Significant Difference         5.9956

               Means with the same letter are not significantly different.

                Tukey Grouping          Mean        N           per

                        A                9.518      8           7
                     B    A              7.157      8           4
                     B    A              5.986      8           6
                     B    A               5.827      8           2
                     B    A              5.431      8           5
                     B    A              5.059      8           3
                     B                   2.902      8           1
```

```
1                        6090NO3              11:18 Monday, October 7, 2002   5

                      The GLM Procedure

         Level of    Level of          --------------V2-------------
         beh         per         N         Mean           Std Dev

         1           1           2      2.7850000       0.3323402
         1           2           2      4.7600000       0.0848528
         1           3           2      4.2700000       1.2869343
         1           4           2      7.1800000       1.0465180
         1           5           2      4.5000000       0.7071068
         1           6           2      4.5000000       0.8626703
         1           7           2      3.0800000       1.1030866
         2           1           2      2.7850000       0.3323402
         2           2           2      8.4800000       2.1778889
         2           3           2      8.2750000       3.3870415
         2           4           2      6.1150000       0.9687363
         2           5           2     10.5100000       4.8931789
         2           6           2     10.8650000       5.9467680
         2           7           2     19.7400000      16.4331616
         3           1           2      3.0200000       0.1979899
         3           2           2      4.6500000       1.2303658
         3           3           2      3.0550000       0.1202082
         3           4           2      7.4150000       3.7547370
         3           5           2      3.3000000       0.1979899
         3           6           2      5.4800000       1.8950462
         3           7           2      5.7300000       0.4949747
         4           1           2      3.0200000       0.1979899
         4           2           2      5.4200000       1.0889444
         4           3           2      4.6350000       0.9687363
         4           4           2      7.9200000       0.0000000
         4           5           2      3.4150000       0.6293250
         4           6           2      3.1000000       3.8749452
         4           7           2      9.5200000       3.3234019
```

APPENDIX 3:

STATISTICAL ANALYSIS OF WINTER TRIAL

Winter trial

Abbreviations

Beh: Behandeling (Treatment)
Per: Periode (Period; Time intervals)
Her: Herhaling (Repetition)

Statistical numbers		Corresponding experimental treatments
Beh	1	Control
	2	Fertilizer
	3	4 ton/ha Sludge
	4	8 ton/ha sludge
	5	16 ton/ha sludge

Statistical numbers		Corresponding experimental days
Per	1	0
	2	10 May
	3	24 May
	4	7 June
	5	21 June
	6	5 July
	7	19 July

Winter trial, 0-30 cm NH_4 levels

```
                              The GLM Procedure
                            Class Level Information
                         Class      Levels          Values
                         beh          5             1 2 3 4 5
                         per          7            1 2 3 4 5 6 7
                         her          2             1 2

                           Number of observations    70

                              The GLM Procedure

Dependent Variable: V1   nh4

                                Sum of
           Source               DF       Squares          Mean Square   F Value      Pr > F
           Model                34     788.538994         23.192323      2.01        0.0216
           Error                 35     403.571400        11.530611
           Corrected Total      69    1192.110394

                     R-Square     Coeff Var     Root MSE      V1 Mean
                     0.661465     115.5665      3.395675     2.938286

           Source              DF     Type I SS      Mean Square   F Value    Pr > F
           beh                  4    59.6771514     14.9192879      1.29    0.2913
           per                  6     84.4845743     14.0807624      1.22    0.3190
           beh*per             24     644.3772686     26.8490529      2.33    0.0111


           Source              DF    Type III SS     Mean Square    F Value    Pr > F
           beh                  4    59.6771514     14.9192879      1.29    0.2913
           per                  6     84.4845743     14.0807624      1.22    0.3190
           beh*per             24     644.3772686     26.8490529      2.33    0.0111
1                                03NH4w                    14:06 Thursday, October 10, 2002   3

                       Alpha                              0.05
                       Error Degrees of Freedom                  35
                       Error Mean Square                  11.53061
                       Critical Value of Studentized Range  4.06595
                       Minimum Significant Difference         3.69

                    Means with the same letter are not significantly different.

                     Tukey Grouping          Mean      N    beh

                          A         4.767      14    3
                          A         2.624      14    5
                          A         2.548      14    1
                          A         2.508      14    2
                          A         2.244      14    4

                       Alpha                              0.05
                       Error Degrees of Freedom                  35
                       Error Mean Square                  11.53061
                       Critical Value of Studentized Range  4.42075
                       Minimum Significant Difference        4.747

                     Tukey Grouping          Mean      N    per

                          A         4.827      10    6
                          A         3.888      10    3
                          A         3.472      10    2
                          A         2.744      10    1
                          A         2.224      10    7
                          A         1.943      10    4
                          A         1.470      10    5
```

```
1                        03NH4w               14:06 Thursday, October 10, 2002   5

                       The GLM Procedure

Level of    Level of              --------------V1-------------
beh         per        N        Mean           Std Dev

1           1          2       3.0800000      2.7718586
1           2          2       3.3600000      4.7517576
1           3          2       3.9700000      3.8890873
1           4          2       1.5400000      0.5939697
1           5          2       2.4350000      0.5161880
1           6          2       0.8700000      1.2303658
1           7          2       2.5800000      1.1879394
2           1          2       2.5200000      2.7718586
2           2          2       1.1200000      0.7919596
2           3          2       1.9600000      0.3959798
2           4          2       4.7600000      4.3557778
2           5          2       2.1000000      2.0930361
2           6          2       1.4550000      1.0253048
2           7          2       3.6400000      1.1879394
3           1          2       2.5200000      2.7718586
3           2          2       2.5200000      1.9798990
3           3          2       5.6000000      5.5437172
3           4          2       0.2800000      0.3959798
3           5          2       1.3150000      0.5161880
3           6          2      20.4100000     13.6613030
3           7          2       0.7250000      0.3181981
4           1          2       3.0800000      2.7718586
4           2          2       4.4800000      0.0000000
4           3          2       2.3100000      1.6829141
4           4          2       1.2300000      0.9475231
4           5          2       0.9400000      0.0141421
4           6          2       0.2250000      0.3181981
4           7          2       3.4450000      2.6516504
5           1          2       2.5200000      2.7718586
5           2          2       5.8800000      7.5236162
5           3          2       5.6000000      2.3758788
5           4          2       1.9050000      1.2657211
5           5          2       0.5600000      0.4808326
5           6          2       1.1750000      0.6293250
5           7          2       0.7300000      0.3959798
```

Winter trial 0-30 cm depth, NO_3 levels

```
                    The GLM Procedure
                 Class Level Information
               Class      Levels    Values
           beh          5               1 2 3 4 5
          per          7              1 2 3 4 5 6 7
             her          2                1 2

               Number of observations    70
Dependent Variable: V2   no3

                          Sum of
 Source                   DF      Squares    Mean Square  F Value      Pr > F
  Model                   34   1227.341680      36.098285     0.88      0.6442
  Error                   35   1435.228800      41.006537
  Corrected Total         69   2662.570480

          R-Square    Coeff Var    Root MSE     V2 Mean
          0.460961     53.38142    6.403635    11.99600

 Source                DF          Type I SS        Mean Square   F Value   Pr > F
 beh                    4          113.5100514      28.3775129     0.69     0.6025
 per                    6          285.3549400      47.5591567     1.16     0.3497
 beh*per               24          828.4766886      34.5198620     0.84     0.6662

 Source                DF   Type III SS    Mean Square   F Value    Pr > F
 beh                    4  113.5100514    28.3775129     0.69      0.6025
 per                    6   285.3549400    47.5591567     1.16      0.3497
 beh*per               24    828.4766886     34.5198620     0.84      0.6662

             Alpha                               0.05
             Error Degrees of Freedom                  35
             Error Mean Square                 41.00654
             Critical Value of Studentized Range  4.06595
             Minimum Significant Difference        6.9586

          Tukey Grouping        Mean      N    beh

                A        14.118    14    3
                A        12.808    14    2
                A        11.245    14    5
                A        10.918    14    1
                A        10.891    14    4

              Alpha                               0.05
             Error Degrees of Freedom                  35
             Error Mean Square                 41.00654
             Critical Value of Studentized Range  4.42075
             Minimum Significant Difference         8.952

          Means with the same letter are not significantly different.


          Tukey Grouping        Mean      N    per

                A        15.842    10    6
                A        13.104    10    1
                A        12.489    10    7
                A        12.200    10    2
                A        11.029    10    3
                A        10.058    10    4
                A         9.250   10    5
```

```
1                          03NO3w              14:06 Thursday, October 10, 2002   5

                             The GLM Procedure

             Level of    Level of            --------------V2-------------
             beh          per        N          Mean           Std Dev

             1            1          2     15.1200000      15.8391919
             1            2          2      3.3600000       0.7919596
             1            3          2     11.7700000       6.3215346
             1            4          2      7.8700000       0.7495332
             1            5          2      9.2950000       4.1931432
             1            6          2     19.2100000      19.5585736
             1            7          2      9.8000000       3.9597980
             2            1          2     11.7600000       7.1276364
             2            2          2     18.4800000      12.6713535
             2            3          2     11.4800000       2.7718586
             2            4          2     10.5000000       2.5738687
             2            5          2      9.4650000       1.4212846
             2            6          2     10.8350000       3.2880465
             2            7          2     17.1350000       0.5586144
             3            1          2     11.7600000       7.1276364
             3            2          2     13.1200000       4.4123463
             3            3          2     10.6400000       3.1678384
             3            4          2     11.4800000       0.3959798
             3            5          2      9.9400000       2.8142850
             3            6          2     28.1650000       5.4659354
             3            7          2     13.7200000       0.2404163
             4            1          2     15.1200000      15.8391919
             4            2          2     14.5600000       0.0000000
             4            3          2     10.8950000       2.7365032
             4            4          2      8.1200000       0.3959798
             4            5          2      6.0950000       0.4596194
             4            6          2     12.7400000       5.0346003
             4            7          2      8.7100000       0.9899495
             5            1          2     11.7600000       7.1276364
             5            2          2     11.4800000       1.1879394
             5            3          2     10.3600000       4.3557778
             5            4          2     12.3200000       0.3959798
             5            5          2     11.4550000       3.0476302
             5            6          2      8.2600000       1.6263456
             5            7          2     13.0800000       1.3859293
```

Winter trial, 30-60 cm depth, NH_4 levels

```
                          The GLM Procedure
                        Class Level Information
                       Class      Levels   Values
                        beh          5   1 2 3 4 5
                       per         7   1 2 3 4 5 6 7
                           her          2   1 2

                       Number of observations    70
                       Dependent Variable: V1   nh4

                       Sum of
Source                 DF       Squares            Mean Square   F Value    Pr > F
 Model                 34       1393.630809        40.989141     2.25       0.0096
 Error                 35       636.894650         18.196990
 Corrected Total       69       2030.525459

            R-Square    Coeff Var    Root MSE     V1 Mean
            0.686340    103.1916     4.265793    4.133857

 Source                DF     Type I SS      Mean Square     F Value    Pr > F
 beh                   4    80.0978514      20.0244629      1.10       0.3717
per                     6   802.5075286      133.7512548      7.35       <.0001
beh*per                24    511.0254286     21.2927262      1.17      0.3297

 Source                DF         Type III SS         Mean Square    F Value    Pr > F
 beh                   4          80.0978514          20.0244629      1.10      0.3717
per                    6          802.5075286         133.7512548      7.35      <.0001
beh*per                24         511.0254286         21.2927262      1.17       0.3297

             Alpha                            0.05
             Error Degrees of Freedom                  35
             Error Mean Square                    18.19699
             Critical Value of Studentized Range  4.06595
             Minimum Significant Difference       4.6355

           Tukey Grouping          Mean         N           beh

                 A                 5.511     14            2
                 A                 5.323     14            5
                 A                  3.576     14           3
                 A                  3.331     14           4
                 A                 2.928     14            1

              Alpha                           0.05
              Error Degrees of Freedom                 35
              Error Mean Square                   18.19699
              Critical Value of Studentized Range  4.42075
              Minimum Significant Difference        5.9634

           Means with the same letter are not significantly different.

            Tukey Grouping          Mean        N           per

                  A                 11.704     10          1
                  B                 5.040      10           3
                  B                 4.760      10          2
                  B                 2.716      10          4
                  B                 1.673      10           7
                  B                 1.624      10          6
                  B                 1.420      10          5
```

```
1                    36NH4w                 14:06 Thursday, October 10, 2002   5

                   The GLM Procedure

  Level of    Level of            --------------V1-------------
  beh         per        N          Mean          Std Dev

  1           1          2        4.4800000      6.3356768
  1           2          2        1.9600000      2.7718586
  1           3          2        4.4800000      4.7517576
  1           4          2        2.5200000      1.9798990
  1           5          2        1.5400000      0.6788225
  1           6          2        0.9500000      0.5515433
  1           7          2        4.5650000      4.3204224
  2           1          2       16.5200000      4.3557778
  2           2          2        6.4400000      7.5236162
  2           3          2        3.3600000      3.1678384
  2           4          2        4.4800000      1.5839192
  2           5          2        1.5100000      0.3959798
  2           6          2        4.9300000      6.8872200
  2           7          2        1.3400000      0.7919596
  3           1          2       16.5200000      4.3557778
  3           2          2        1.6800000      1.5839192
  3           3          2        2 8000000      0.7919596
  3           4          2        1.7350000      0.8697413
  3           5          2        1.2050000      1.3081475
  3           6          2        0.6150000      0.8697413
  3           7          2        0.4750000      0.2757716
  4           1          2        4.4800000      6.3356768
  4           2          2        8.9600000      0.0000000
  4           3          2        3.0800000      2.7718586
  4           4          2        2.4100000      1.8243355
  4           5          2        1.5100000      0.5515433
  4           6          2        1.0900000      0.0424264
  4           7          2        1.7900000      0.7919596
  5           1          2       16.5200000      4.3557778
  5           2          2        4.7600000      6.7316566
  5           3          2       11.4800000     16.2351717
  5           4          2        2.4350000      0.1202082
  5           5          2        1.3350000      1.1808683
  5           6          2        0.5350000      0.1202082
  5           7          2        0.1950000      0.1202082
```

Winter trial, 30-60cm NO_3 levels

```
                         The GLM Procedure
                      Class Level Information
                     Class        Levels    Values
                      beh            5    1 2 3 4 5
                     per           7    1 2 3 4 5 6 7
                         her           2    1 2

                     Number of observations    70

Dependent Variable: V2   no3
                                    Sum of
          Source               DF      Squares         Mean Square   F Value      Pr > F
           Model               34      2606.600357     76.664716     1.51         0.1165
           Error               35      1781.476850     50.899339
           Corrected Total     69      4388.077207

                R-Square    Coeff Var    Root MSE     V2 Mean
                0.594019    44.30307     7.134377    16.10357

          Source               DF        Type I SS     Mean Square   F Value      Pr > F
           beh                 4         330.210614    82.552654     1.62         0.1906
          per                  6        1206.357477    201.059580     3.95        0.0040
          beh*per              24       1070.032266    44.584678     0.88         0.6276

           Source             DF   Type III SS    Mean Square   F Value   Pr > F
           beh                 4    330.210614     82.552654     1.62    0.1906
          per                6    1206.357477    201.059580     3.95    0.0040
          beh*per             24    1070.032266     44.584678     0.88    0.6276
1                             36NO3w                     14:06 Thursday, October 10, 2002   3

                   Alpha                          0.05
                   Error Degrees of Freedom                35
                   Error Mean Square               50.89934
                   Critical Value of Studentized Range  4.06595
                   Minimum Significant Difference       7.7527

                 Means with the same letter are not significantly different.

                 Tukey Grouping        Mean     N    beh

                       A       18.981    14    2
                       A       18.201    14    5
                       A       15.656    14    3
                       A       14.319    14    4
                       A       13.361    14    1


                   Alpha                          0.05
                   Error Degrees of Freedom                35
                   Error Mean Square               50.89934
                   Critical Value of Studentized Range  4.42075
                   Minimum Significant Difference       9.9736

                 Means with the same letter are not significantly different.

                  Tukey Grouping        Mean     N    per

                        A       22.288    10    1
                     B   A       20.272    10    3
                     B   A       18.166    10    7
                     B   A       16.856    10    2
                     B   A       12.718    10    6
                     B          11.878    10    4
                     B          10.547    10    5
```

```
1                        36NO3w              14:06 Thursday, October 10, 2002   5

                     The GLM Procedure

        Level of    Level of          -------------V2-------------
        beh         per         N        Mean           Std Dev

        1           1           2     17.9200000      5.5437172
        1           2           2     13.4400000     11.0874343
        1           3           2     10.9200000      5.9396970
        1           4           2     13.1900000      4.3133514
        1           5           2      7.3950000      1.2657211
        1           6           2     12.1250000      7.2478445
        1           7           2     18.5350000      1.5061374
        2           1           2     25.2000000      3.1678384
        2           2           2     31.0800000     10.6914545
        2           3           2     18.7600000      0.3959798
        2           4           2     10.6400000      3.9597980
        2           5           2     12.6850000      0.8273149
        2           6           2     12.0700000      1.6263456
        2           7           2     22.4300000      5.5861436
        3           1           2     25.2000000      3.1678384
        3           2           2     15.1200000      7.9195959
        3           3           2     16.5200000      3.5638182
        3           4           2     11.0900000      1.4283557
        3           5           2      9.6600000      0.7495332
        3           6           2     17.8350000     11.6036223
        3           7           2     14.1650000      0.8697413
        4           1           2     17.9200000      5.5437172
        4           2           2     12.8800000      0.0000000
        4           3           2     26.8800000     19.0070303
        4           4           2     11.6200000      2.5738687
        4           5           2      7.5300000      0.1979899
        4           6           2     10.1900000      4.5961941
        4           7           2     13.2150000      5.3103719
        5           1           2     25.2000000      3.1678384
        5           2           2     11.7600000      7.1276364
        5           3           2     28.2800000     20.9869293
        5           4           2     12.8500000      7.4811897
        5           5           2     15.4650000      4.4194174
        5           6           2     11.3700000      2.2203153
        5           7           2     22.4850000      9.4681598
```

Winter trial, 60-90cm depth, NH_4 levels

```
                          The GLM Procedure
                       Class Level Information
                     Class       Levels    Values
                      beh            5    1 2 3 4 5
                     per           7    1 2 3 4 5 6 7
                        her            2    1 2

                     Number of observations    70

        NOTE: Due to missing values, only 63 observations can be used in this analysis.


Dependent Variable: V1   nh4
                                  Sum of
           Source               DF      Squares          Mean Square   F Value   Pr > F
            Model               31      664.179710       21.425152     1.96      0.0323
            Error               31      338.116950       10.906998
            Corrected Total     62      1002.296660

                    R-Square    Coeff Var    Root MSE     V1 Mean
                    0.662658    114.6411     3.302575     2.880794

           Source             DF     Type I SS     Mean Square   F Value   Pr > F
            beh                4    83.1803866     20.7950966     1.91    0.1343
           per                 6   238.3248617     39.7208103     3.64    0.0075
           beh*per            21    342.6744621     16.3178315     1.50   0.1507

            Source             DF    Type III SS    Mean Square   F Value   Pr > F
            beh                4    92.0383931     23.0095983     2.11    0.1034
           per                 6   232.1678881     38.6946480     3.55    0.0086
           beh*per            21    342.6744621     16.3178315     1.50   0.1507
1                              69NH4w                 14:06 Thursday, October 10, 2002   3

                NOTE: This test controls the Type I experimentwise error rate.

                     Alpha                          0.05
                     Error Degrees of Freedom             31
                     Error Mean Square                10.907
                     Critical Value of Studentized Range  4.09389

                Comparisons significant at the 0.05 level are indicated by ***
                                 Difference     Simultaneous
                     beh          Between    95% Confidence
                   Comparison        Means        Limits

                   4    - 3        0.068     -3.546   3.681
                   4    - 2        1.206     -3.031   5.443
                   4    - 5        2.296     -1.386   5.978
                   4    - 1        2.652     -0.961   6.266
                   3    - 4       -0.068     -3.681   3.546
                   3    - 2        1.138     -3.099   5.375
                   3    - 5        2.228     -1.454   5.910
                   3    - 1        2.584     -1.029   6.198
                   2    - 4       -1.206     -5.443   3.031
                   2    - 3       -1.138     -5.375   3.099
                   2    - 5        1.090     -3.206   5.386
                   2    - 1        1.446     -2.791   5.683
                   5    - 4       -2.296     -5.978   1.386
                   5    - 3       -2.228     -5.910   1.454
                   5    - 2       -1.090     -5.386   3.206
                   5    - 1        0.356     -3.326   4.038
                   1    - 4       -2.652     -6.266   0.961
                   1    - 3       -2.584     -6.198   1.029
                   1    - 2       -1.446     -5.683   2.791
                   1    - 5       -0.356     -4.038   3.326
```

```
    Alpha                          0.05
   Error Degrees of Freedom             31
   Error Mean Square               10.907
   Critical Value of Studentized Range  4.45433

Comparisons significant at the 0.05 level are indicated by ***

              Difference    Simultaneous
    per         Between     95% Confidence
  Comparison        Means        Limits

  2   - 1         1.331    -3.321  5.983
  2   - 3         1.752    -2.900  6.404
  2   - 6         4.259    -0.676  9.193
  2   - 7         4.730    -0.397  9.856
  2   - 5         4.734    -0.201  9.668
  2   - 4         4.873     0.221  9.525  ***
  1   - 2        -1.331    -5.983  3.321
  1   - 3         0.421    -4.231  5.073
  1   - 6         2.927    -2.007  7.862
  1   - 7         3.399    -1.728  8.525
  1   - 5         3.402    -1.532  8.337
  1   - 4         3.542    -1.110  8.194
  3   - 2        -1.752    -6.404  2.900
  3   - 1        -0.421    -5.073  4.231
  3   - 6         2.506    -2.428  7.441
  3   - 7         2.978    -2.149  8.104
  3   - 5         2.981    -1.953  7.916
  3   - 4         3.121    -1.531  7.773
  6   - 2        -4.259    -9.193  0.676
  6   - 1        -2.927    -7.862  2.007
  6   - 3        -2.506    -7.441  2.428
  6   - 7         0.471    -4.913  5.855
  6   - 5         0.475    -4.726  5.676
  6   - 4         0.614    -4.320  5.549
  7   - 2        -4.730    -9.856  0.397
  7   - 1        -3.399    -8.525  1.728
  7   - 3        -2.978    -8.104  2.149
  7   - 6        -0.471    -5.855  4.913
  7   - 5         0.004    -5.380  5.388
  7   - 4         0.143    -4.983  5.270
  5   - 2        -4.734    -9.668  0.201
  5   - 1        -3.402    -8.337  1.532
  5   - 3        -2.981    -7.916  1.953
  5   - 6        -0.475    -5.676  4.726
  5   - 7        -0.004    -5.388  5.380
  5   - 4         0.139    -4.795  5.074
  4   - 2        -4.873    -9.525 -0.221  ***
  4   - 1        -3.542    -8.194  1.110

1                        69NH4w              14:06 Thursday, October 10, 2002   5

                          The GLM Procedure

                Tukey's Studentized Range (HSD) Test for V1

Comparisons significant at the 0.05 level are indicated by ***.

              Difference    Simultaneous
    per         Between     95% Confidence
  Comparison        Means        Limits
   4   - 3       -3.121    -7.773  1.531
   4   - 6       -0.614    -5.549  4.320
   4   - 7       -0.143    -5.270  4.983
   4   - 5       -0.139    -5.074  4.795
```

```
1                          69NH4w                 14:06 Thursday, October 10, 2002   6
                        The GLM Procedure
           Level of    Level of          --------------V1-------------
           beh          per        N          Mean         Std Dev

           1            1          2     1.9600000      1.97989899
           1            2          2     1.3750000      0.95459415
           1            3          2     2.2400000      1.58391919
           1            4          2     1.1200000      1.58391919
           1            5          2     1.1650000      0.09192388
           1            6          2     0.3650000      0.03535534
           1            7          2     1.9950000      1.87383297
           2            1          2     6.1600000      7.12763635
           2            2          2     1.7600000      0.90509668
           2            3          2     2.6550000      0.99702056
           2            4          2     1.0500000      0.09899495
           3            1          2     6.1600000      7.12763635
           3            2          2     9.2400000      1.97989899
           3            3          2     5.8800000      5.93969696
           3            4          2     1.1500000      0.04242641
           3            5          2     1.1800000      0.39597980
           3            6          2     3.8600000      3.16783838
           3            7          2     0.8400000      0.70710678
           4            1          2     1.9600000      1.97989899
           4            2          2    15.6800000       0.00000000
           4            3          2     7.0000000      9.89949494
           4            4          2     0.8400000      1.18793939
           4            5          2     1.3200000      1.21622366
           4            6          2     1.5950000      2.25567063
           4            7          2     0.3900000      0.15556349
           5            1          2     6.1600000      7.12763635
           5            2          2     1.0000000      1.41421356
           5            3          2     2.5200000      2.77185858
           5            4          2     0.5300000      0.35355339
           5            5          2     0.6450000      0.91216775
           5            6          2     0.3900000      0.24041631
           5            7          1     1.1200000       .
```

Winter trial, 60-90 cm depth NO_3 levels

```
                              The GLM Procedure
                           Class Level Information
                          Class      Levels    Values
                           beh           5    1 2 3 4 5
                          per          7   1 2 3 4 5 6 7
                              her           2    1 2

                         Number of observations    70

NOTE: Due to missing values, only 63 observations can be used in this analysis.
Dependent Variable: V2   no3

                            Sum of
        Source             DF       Squares          Mean Square   F Value   Pr > F
         Model             31       3458.235427      111.555982    3.07      0.0012
         Error             31       1125.127100      36.294423
         Corrected Total   62       4583.362527

               R-Square   Coeff Var    Root MSE     V2 Mean
               0.754519   33.40279     6.024485    18.03587

         Source            DF       Type 1 SS        Mean Square   F Value   Pr > F
         beh               4        597.893718       149.473430    4.12      0.0086
        per                6        505.327539       84.221257     2.32      0.0576
        beh*per            21       2355.014170      112.143532    3.09      0.0022

         Source          DF    Type III SS    Mean Square    F Value    Pr > F
         beh             4    1057.774453    264.443613      7.29    0.0003
        per            6    979.845420    163.307570     4.50    0.0022
        beh*per          21    2355.014170    112.143532     3.09    0.0022
1                        69NO3w                     14:06 Thursday, October 10, 2002   3

                  Alpha                         0.05
                  Error Degrees of Freedom          31
                  Error Mean Square             36.29442
                  Critical Value of Studentized Range  4.09389

            Comparisons significant at the 0.05 level are indicated by ***.

                      Difference    Simultaneous
                beh     Between     95% Confidence
             Comparison     Means       Limits

             5   - 2      3.229    -4.608  11.065
             5   - 1      5.095    -1.622  11.812
             5   - 3      6.310    -0.407  13.027
             5   - 4      8.999     2.281  15.716  ***
             2   - 5     -3.229   -11.065   4.608
             2   - 1      1.866    -5.863   9.596
             2   - 3      3.081    -4.648  10.811
             2   - 4      5.770    -1.960  13.499
             1   - 5     -5.095   -11.812   1.622
             1   - 2     -1.866    -9.596   5.863
             1   - 3      1.215    -5.377   7.807
             1   - 4      3.904    -2.688  10.495
             3   - 5     -6.310   -13.027   0.407
             3   - 2     -3.081   -10.811   4.648
             3   - 1     -1.215    -7.807   5.377
             3   - 4      2.689    -3.903   9.280
             4   - 5     -8.999   -15.716  -2.281  ***
             4   - 2     -5.770   -13.499   1.960
             4   - 1     -3.904   -10.495   2.688
             4   - 3     -2.689    -9.280   3.903
1                        69NO3w                     14:06 Thursday, October 10, 2002   4
```

```
                    The GLM Procedure
           Tukey's Studentized Range (HSD) Test for V2
    NOTE: This test controls the Type I experimentwise error rate

          Alpha                              0.05
          Error Degrees of Freedom                   31
          Error Mean Square                    36.29442
          Critical Value of Studentized Range  4.45433

   Comparisons significant at the 0.05 level are indicated by ***.

                     Difference     Simultaneous
          per         Between       95% Confidence
       Comparison       Means          Limits
        7   - 1         4.151     -5.200  13.502
        7   - 2         4.427     -4.924  13.778
        7   - 3         5.318     -4.033  14.669
        7   - 4         6.480     -2.871  15.831
        7   - 6         7.890     -1.931  17.710
        7   - 5         9.883      0.063  19.704  ***
        1   - 7        -4.151    -13.502   5.200
        1   - 2         0.276     -8.210   8.762
        1   - 3         1.167     -7.319   9.653
        1   - 4         2.329     -6.157  10.815
        1   - 6         3.739     -5.262  12.739
        1   - 5         5.732     -3.269  14.733
        2   - 7        -4.427    -13.778   4.924
        2   - 1        -0.276     -8.762   8.210
        2   - 3         0.891     -7.595   9.377
        2   - 4         2.053     -6.433  10.539
        2   - 6         3.463     -5.538  12.463
        2   - 5         5.456     -3.545  14.457
        3   - 7        -5.318    -14.669   4.033
        3   - 1        -1.167     -9.653   7.319
        3   - 2        -0.891     -9.377   7.595
        3   - 4         1.162     -7.324   9.648
        3   - 6         2.571     -6.429  11.572
        3   - 5         4.565     -4.436  13.566
        4   - 7        -6.480    -15.831   2.871
        4   - 1        -2.329    -10.815   6.157
        4   - 2        -2.053    -10.539   6.433
        4   - 3        -1.162     -9.648   7.324
        4   - 6         1.409     -7.591  10.410
        4   - 5         3.403     -5.598  12.404
        6   - 7        -7.890    -17.710   1.931
        6   - 1        -3.739    -12.739   5.262
        6   - 2        -3.463    -12.463   5.538
        6   - 3        -2.571    -11.572   6.429
        6   - 4        -1.409    -10.410   7.591
        6   - 5         1.994     -7.494  11.481
        5   - 7        -9.883    -19.704  -0.063  ***
        5   - 1        -5.732    -14.733   3.269
        5   - 2        -5.456    -14.457   3.545
        5   - 3        -4.565    -13.566   4.436
        5   - 4        -3.403    -12.404   5.598
        5   - 6        -1.994    -11.481   7.494
1                       69NO3w                  14:06 Thursday, October 10, 2002   6

                    The GLM Procedure


    Level of    Level of          --------------V2-------------
    beh          per      N        Mean           Std Dev

    1            1        2     15.6800000      11.0874343
    1            2        2     30.5200000       3.5638182
    1            3        2     13.1600000       6.7316566
```

1	4	2	16.1300000	3.3234019
1	5	2	12.5500000	3.7193817
1	6	2	16.2400000	3.0122749
1	7	2	20.9150000	9.3833070
2	1	2	21.8400000	0.0000000
2	2	2	16.5200000	7.5236162
2	3	2	25.2450000	14.9835927
2	4	2	15.4000000	1.9798990
3	1	2	21.8400000	0.0000000
3	2	2	15.4400000	3.6203867
3	3	2	15.9600000	4.3557778
3	4	2	16.9400000	3.7618081
3	5	2	14.3950000	7.9973777
3	6	2	18.3400000	12.3885108
3	7	2	13.7750000	2.6940768
4	1	2	15.6800000	11.0874343
4	2	2	15.1200000	0.0000000
4	3	2	15.4000000	2.7718586
4	4	2	17.4450000	5.4235090
4	5	2	10.9850000	2.1566757
4	6	2	8.7350000	1.3505740
4	7	2	14.5050000	2.4536605
5	1	2	21.8400000	0.0000000
5	2	2	17.9000000	1.5556349
5	3	2	21.2800000	8.7115555
5	4	2	19.3200000	1.5839192
5	5	2	16.6450000	1.0111627
5	6	2	19.2350000	3.4436100
5	7	1	66.3000000	.

APPENDIX 4:

STATISTICAL ANALYSIS OF DENITRIFICATION TRIAL

N_2O trial

Abbreviations

Beh: Behandeling (Treatment)
Per: Periode (Period; Time intervals)
Her: Herhaling (Repetition)

N2O100 = N_2O production at 100% FC
N2O50 = N_2O production at 50% FC

Statistical numbers		**Corresponding experimental treatments**
Beh	1	10 ton/ha sludge
	2	20 ton/ha sludge
	3	10 ton/ha fertilizer
	4	20 ton/ha fertilizer
	5	Control

Statistical numbers		**Corresponding experimental days**
Per	1	1
	2	3
	3	7
	4	14
	5	21
	6	28

```
1                             N2O100                15:04 Thursday, June 19, 2003   1
                         The GLM Procedure
                      Class Level Information
                     Class        Levels    Values
                     beh             5    1 2 3 4 5
                     per            6    1 2 3 4 5 6
                       herh            3    1 2 3

                         The GLM Procedure
                     Dependent Variable: v1   N2O

                                 Sum of
            Source           DF       Squares     Mean Square   F Value    Pr > F
            Model            29    4841.166579     166.936779     19.34    <.0001
                  Error            60     517.795497       8.629925
                    Corrected Total         89    5358.962077

                 R-Square    Coeff Var     Root MSE      v1 Mean
                 0.903378     83.55026     2.937673     3.516056

        Source             DF       Type I SS           Mean Square      F Value     Pr > F

        Beh                4         1613.703500       403.425875        46.75      <.0001
        per                5        1259.143006       251.828601        29.18      <.0001
        beh*per            20       1968.320073       98.416004         11.40      <.0001

         Source             DF     Type III SS          Mean Square      F Value     Pr > F

        beh                4        1613.703500      403.425875         46.75     <.0001
        per                5        1259.143006      251.828601         29.18     <.0001
        beh*per            20       1968.320073       98.416004         11.40     <.0001
                  N2O100                     15:04 Thursday, June 19, 2003   3
                                The GLM Procedure
                         Tukey's Studentized Range (HSD) Test for v1

                            Alpha                            0.05
                         Error Degrees of Freedom                60
                         Error Mean Square                 8.629925
                       Critical Value of Studentized Range   3.97742
                        Minimum Significant Difference         2.754

                     Means with the same letter are not significantly different.

            Tukey       Grouping           Mean       N          beh

                          A                10.1731     18         3
                          B                6.9204      18         2
                          C                0.1791      18         4
                          C                0.1588      18         1
                          C                0.1489      18         5
1                          N2O100                    15:04 Thursday, June 19, 2003   4

                          The GLM Procedure

                    Tukey's Studentized Range (HSD) Test for v1

   NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than
REGWQ.
```

```
          Alpha                          0.05
          Error Degrees of Freedom                60
          Error Mean Square               8.629925
          Critical Value of Studentized Range  4.16317
          Minimum Significant Difference      3.1578


      Means with the same letter are not significantly different.


       Tukey Grouping          Mean     N    per

                  A            10.411    15    1
                  B             6.607    15    2
                  C             2.005    15    3
                  C             1.313    15    4
                  C             0.529    15    5
                  C             0.231    15    6

1                         N2O100                    15:04 Thursday, June 19, 2003   5

                      The GLM Procedure

      Level of    Level of            --------------v1-------------
     beh          per        N          Mean           Std Dev

     1           1          3       0.2890000       0.0752861
     1           2          3       0.0706667       0.0132035
     1           3          3       0.1426667       0.0453468
     1           4          3       0.1020000       0.0266271
     1           5          3       0.1883333       0.0645316
     1           6          3       0.1600000       0.0301993
     2           1          3      21.0416667        2.6845950
     2           2          3      13.9396667        4.8815957
     2           3          3       2.9696667       1.4858130
     2           4          3       1.8143333       1.0061175
     2           5          3       0.9156667       0.5426844
     2           6          3       0.8413333       0.4001454
     3           1          3      30.3423333      14.1668494
     3           2          3      18.7396667        3.5705938
     3           3          3       6.5000000       1.1789826
     3           4          3       4.2050000       2.9920707
     3           5          3       1.2516667       0.5840842
     3           6          3       0.0000000       0.0000000
     4           1          3       0.2563333       0.0289194
     4           2          3       0.1816667       0.1097695
     4           3          3       0.2293333       0.0384751
     4           4          3       0.2266667       0.0882345
     4           5          3       0.1046667       0.0429108
     4           6          3       0.0756667       0.0205508
     5           1          3       0.1270000       0.0802994
     5           2          3       0.1013333       0.0290230
     5           3          3       0.1823333       0.0162583
     5           4          3       0.2176667       0.0856874
     5           5          3       0.1856667       0.0136137
     5           6          3       0.0796667       0.0245425
```

```
                          N2O50  15:04 Thursday, June 19, 2003   1

                                 The GLM Procedure
                              Class Level Information
                             Class       Levels   Values
                              beh           3     1 3 5
                             per           4    1 2 3 4
                               vog            2    1 2
                              herh          3    1 2 3

                            Number of observations    72

1                            N2O50                 15:04 Thursday, June 19, 2003   2

                           The GLM Procedure
 Dependent Variable: v1   N2O

                                   Sum of
          Source                     DF       Squares         Mean Square      F Value    Pr > F
           Model                     23       6023.901363     261.908755       21.86     <.0001
           Error                     48       575.215109     11.983648
           Corrected Total           71       6599.116471

                 R-Square     Coeff Var      Root MSE       v1 Mean
                  0.912835     75.18010      3.461741      4.604597

          Source             DF       Type I SS          Mean Square    F Value     Pr > F

          beh                2        2835.554139     1417.777069        118.31    <.0001
          per                3        1017.954025      339.318008        28.32    <.0001
          beh*per            6        2028.021504      338.003584        28.21    <.0001
          vog                1        16.614730          16.614730        1.39    0.2448
          beh*vog            2        35.055284      17.527642            1.46    0.2418
          per*vog            3        29.145589       9.715196            0.81    0.4942
          beh*per*vog         6        61.556093      10.259349           0.86    0.5337

          Source             DF     Type III SS          Mean Square    F Value     Pr > F

          beh                2        2835.554139     1417.777069    118.31    <.0001
          per                3       1017.954025      339.318008     28.32     <.0001
          beh*per            6        2028.021504      338.003584     28.21     <.0001
          vog                1       16.614730      16.614730        1.39       0.2448
          beh*vog            2       35.055284      17.527642        1.46        0.2418
          per*vog            3        29.145589       9.715196       0.81       0.4942
          beh*per*vog        6        61.556093      10.259349        0.86       0.5337


                           The GLM Procedure

                Tukey's Studentized Range (HSD) Test for v1

    NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than
 REGWQ.


                Alpha                          0.05
                Error Degrees of Freedom              48
                Error Mean Square                11.98365
                Critical Value of Studentized Range  3.42026
                Minimum Significant Difference       2.4168
```

```
              Means with the same letter are not significantly different.

               Tukey Grouping        Mean          N        beh

                     A             13.4796         24        3
                     B             0.1704          24        5
                     B             0.1638          24        1

    NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than
REGWQ.

                   Alpha                          0.05
                   Error Degrees of Freedom                48
                   Error Mean Square                11.98365
                   Critical Value of Studentized Range  3.76375
                   Minimum Significant Difference        3.071

               Means with the same letter are not significantly different.

               Tukey Grouping        Mean           N        per

                     A             10.632           18        1
                     B             4.904            18        2
                     C             1.694            18        3
                     C             1.188            18        4

              beh       per       N         Mean          Std Dev

              1         1         6      0.2293333     0.0829233
              1         2         6      0.1286667     0.0668032
              1         3         6      0.1555000     0.0579370
              1         4         6      0.1418333     0.0471865
              3         1         6     31.5038333    10.1376576
              3         2         6     14.4615000     5.3654365
              3         3         6      4.7500000     2.6105213
              3         4         6      3.2030000     2.2612623
              5         1         6      0.1613333     0.0818063
              5         2         6      0.1221667     0.0428365
              5         3         6      0.1775000     0.0174900
              5         4         6      0.2205000     0.0588821

                   Alpha                          0.05
                   Error Degrees of Freedom                48
                   Error Mean Square                11.98365
                   Critical Value of Studentized Range  2.84352
                   Minimum Significant Difference       1.6406


               Means with the same letter are not significantly different.

                Tukey Grouping              Mean      N        vog

                      A                    5.0850     36        1
                      A                    4.1242     36        2
```

```
1                    N2O50              15:04 Thursday, June 19, 2003   7

                    The GLM Procedure


          Level of    Level of          --------------v1-------------
          beh         vog       N         Mean          Std Dev

          1           1         12      0.1510833      0.0958782
          1           2         12      0.1765833      0.0382253
          3           1         12     14.9467500     12.6563367
          3           2         12     12.0124167     13.3132764
          5           1         12      0.1570833      0.0705542
          5           2         12      0.1836667      0.0542039


          Level of    Level of          --------------v1-------------
          per         vog       N         Mean          Std Dev

          1           1         9      10.2527778     16.6493954
          1           2         9      11.0102222     16.6381723
          2           1         9       6.3038889      9.4961853
          2           2         9       3.5043333      5.1158481
          3           1         9       2.2750000      3.2232517
          3           2         9       1.1136667      1.9020981
          4           1         9       1.5082222      2.5166419
          4           2         9       0.8686667      1.0972092


       Level of    Level of    Level of          --------------v1-------------
       beh         per         vog       N         Mean          Std Dev

       1           1           1         3       0.2890000      0.0752861
       1           1           2         3       0.1696667      0.0290230
       1           2           1         3       0.0706667      0.0132035
       1           2           2         3       0.1866667      0.0298385
       1           3           1         3       0.1426667      0.0453468
       1           3           2         3       0.1683333      0.0764286
       1           4           1         3       0.1020000      0.0266271
       1           4           2         3       0.1816667      0.0098658
       3           1           1         3      30.3423333     14.1668494
       3           1           2         3      32.6653333      7.2238069
       3           2           1         3      18.7396667      3.5705938
       3           2           2         3      10.1833333      2.0766560
       3           3           1         3       6.5000000      1.1789826
       3           3           2         3       3.0000000      2.5415651
       3           4           1         3       4.2050000      2.9920707
       3           4           2         3       2.2010000      0.9048586
       5           1           1         3       0.1270000      0.0802994
       5           1           2         3       0.1956667      0.0821361
       5           2           1         3       0.1013333      0.0290230
       5           2           2         3       0.1430000      0.0494267
       5           3           1         3       0.1823333      0.0162583
       5           3           2         3       0.1726667      0.0207445
       5           4           1         3       0.2176667      0.0856874
       5           4           2         3       0.2233333      0.0360740
```