Influence of biosolid stability, temperature and water potential on nitrogen mineralisation in biosolid amended soils

by

Laurinda Nobela

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Supervisor: P.C. de Jager
Co-Supervisor: J.G. Annandale

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DECLARATION

I hereby certify that this thesis I am submitting to the University of Pretoria for the degree, MSc. (Agric) Soil Science, is entirely my own work, except where duly acknowledged. I also certify that this thesis has never been submitted to any other tertiary institution for any degree.

Signature ______________________________ Date: _________________________

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Rest in peace in God's hands!

Summary

Soils with inherently low soil fertility, and nutrient depletion of fertile soils, are the root causes of declining per capita food production in Africa. On the other hand, demand for better water quality and strict environmental laws have led to an increase in biosolid production. Accumulation of this waste poses an increasing environmental pollution risk. Disposal methods like incineration, ocean dumping and land filling are causing enormous environmental and economic problems. Therefore, municipal authorities have been challenged with the environmental management of biosolids, whilst many farmers are facing a problem of soil fertility decline. Biosolids of “Exceptional quality class A” contain high organic matter, plant nutrients and have few restrictions on use for land application. Therefore, it is a valuable resource. Beneficiation of sewage sludge through land application is an optional solution to address both soil fertility and environmental problems. Scientific management of sewage sludge utilization must be observed to minimize environmental problems. The study of N release and the rate of nitrification from biosolids is essential to improve nutrient use efficiency, as well as to prevent environmental pollution. Mineralization and nitrification processes are influenced by several factors, for instance, the origin and quality of organic material, and soil environmental conditions, of which moisture and temperature are the most important factors. The study aims to: (i) evaluate biosolid stability, temperature and soil water effects on net N release from municipal and industrial sludge amended soil, and (ii) generate important parameters for modeling N dynamics (rate constants and half life). This dissertation consists of two major experiments: The first experiment was a fifty six day laboratory incubation study to assess N release and nitrification rate constants in a biosolid amended soil, as well as the biosolid’s half life time. The experiment was conducted using three types of biosolids originated from three different wastewater treatment processes, subjected to three levels of temperature and three of soil water potentials. The second experiment was an investigation on sample handling strategy for accurate nitrate (NO_3^-) and ammonium (NH_4^+) determinations. Different handling procedures: Direct field extraction, Field drying extraction and Laboratory drying extraction were tested on biosolid amended soils. In conclusion, biosolid stability, temperature and soil water interaction significantly influence mineralization and nitrification processes. Unstable sludges had higher N mineralization rate constant and shorter half life times compared to stable sludge, and the Direct field extraction procedure proved to be the most representative sample handling strategy for determination of N speciation in soils and biosolid amended soils to get representative time specific data.

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Abbreviations

CIAT- Centro Internacional para Agricultura Tropical/ International Centre for Tropical Agriculture

DEAT- Department of Environmental Affairs and Tourism

DEF- Direct Field Extraction

DoA- Department of Agriculture

DoH- Department of Health

DWAF- Department of Water Affairs and Forestry

EUUWTD- European Union Urban Wastewater Treatment Directive

ERWAT- East Rand Water Care Company

FC- field capacity

FDE- Field Dried Extraction

ICRAF- International Centre for Agro-forestry research

LDE- Laboratory Dried Extraction

NMIT- Nitrogen mineralization immobilization turnover

SASOL- Suid Afrikaanse Steenkool en Olie Maatskappy / South African Coal and Oil Company

SOM- Soil organic matter

SMB- Soil microbe biomass

TSBF- Tropical Soil Biology and Fertility programme

UK- United Kingdom

USA- United States of America

USEPA- United States Environmental Protection Agency

WWTP- Waste water treatment plant

CHAPTER I: GENERAL INTRODUCTION

1.1 Soil fertility decline

Soil fertility is the status of a soil that gives an indication of its potential to supply plant nutrients. Its evaluation is based on soil physical and chemical properties. It varies with time, place, and agricultural use. Fertile soil, when located in an agro-ecological region suitable for crop growth, is considered potentially productive.

Many agricultural lands are continuously losing their productivity as a result of soil fertility decline and/or as a result of utilization of soils with inherent low soil fertility (Folmer *et al.*, 1998; Dowgill *et al.*, 2002). Decrease in soil productivity has been observed in more than 10 % of cultivated land worldwide, since the 1980's (Burns *et al.*, 2006; Francavigilia, 2004; CIAT, ICRAF and TSBF, 2002).

Low levels of food production in Africa results from intensive extraction of plant nutrients without any replenishing measures (Stoorvogel and Smaling, 1990; Buresh *et al.*, 1997; Scoones, 2001). In Kenya, according to Smaling (1993) most forest and grassland soils showed a significant decline in fertility after being cleared and cultivated continuously with no replenishment of nutrients.

The removal of soil nutrients by crops was greatly exceeding any inputs as a result of insufficient fallow period to recycle back plant nutrients, or in areas of continuous cultivation in, sub-Saharan Africa, (Smaling, 1993). Negative nutrient balance for N, P, and K in several East and Southern Africa studied soils was evident (Stoorvogel and Smaling, 1990; Stoorvogel *et al.*, 1993; Dowgill *et al.*, 2002).

The evidence of soil fertility degradation is the manifestation of plant nutrient deficiencies, low soil organic matter content and higher soil erodibility. Along with erosion, nutrient depletion represents the major land degradation threats in Southern Africa.

Nitrogen (N) and phosphorus (P) are the essential macro-nutrients often limiting crop production, and can be supplied through applications of inorganic fertilizers. However, due to economic reasons most farmers cannot afford to purchase mineral fertilizers (Waddington, 2003; Nhemachena *et al.*, 2003). Therefore the use of locally available organic sources of plant nutrients is a valuable alternative for the maintenance and recovery of soil fertility.

The Rockefeller Foundation hosted a workshop in March 2002, with the purpose to create a forum to address issues related to recovery measures of soil fertility decline. Various international institutes (International center for tropical agriculture-CIAT, International center for agro-forestry research-ICRAF and Tropical soil biology and fertility programme- TSBF) joined their efforts to find solutions for combating nutrient depletion.

An integrated natural resource management concept was proposed to steer the research related to soil fertility recovery. This concept resides in the utilization of locally available natural resources in both an economical and environmentally sustainable way. In agricultural lands nutrients exported by the crops need to be replaced through the addition of readily available sources of nutrients (cost effective) and sustainable agricultural practices management should be implemented (environmental friendly).

Therefore from an African perspective soil fertility management research should focus on ways to increase crop production with minimal use of inorganic fertilizers, and supplementing with organic sources such as animal manure, crop residues, legume based green manure, municipal and industrial wastes, and etc (CIAT, ICRAF and TSBF, 2002; Rowe and Giller, 2003).

1.2 Trends in sewage sludge disposal

Sewage sludge is a by-product of water care works plants, rich in organic matter and plant nutrients. It is a possible organic source that can be utilized in urban and peri-urban areas, in South Africa and other African cities where water care works does exist.

Ever increasing volume of sewage sludge is produced as a result of the growing human population on earth. Additionally, better water quality is being demanded and stricter environmental laws prescribed, thereby also contributing to an increase in sewage sludge production.

Accumulation of produced sewage sludge is a problem due to its negative sanitary status and polluting effect. As a result sewage sludge disposal became a global challenge (Peverly, 1996; Smith, 1996; Walter *et al*., 2006). The situation in South Africa with regards to sewage sludge production also reflects this global trend.

1.2.1 Disposal strategies

In the past incineration, ocean dumping and land filling at sacrificial site were common sewage sludge disposal strategies. Sewage sludge disposal through ocean dumping was banned in the USA in 1991. This practice was also banned in Europe in 1998 through the implementation of the EUUWTD- 91/271/EC (European Union Urban Wastewater Treatment Directive. High energy requirement limits incineration and scarcity of land resource also reduces land filling as a disposal option. Sewage sludge disposal, through land application is increasingly seen as a viable strategy (Mc Grath *et al*., 1994; Peverly, 1996; Snyman *et al*., 1998; Kelly *et al*., 1999; Bowler, 1999; Debosz *et al*., 2002; IWA, 2003; Bengtsson and Tillman, 2004; Van Niekerk *et al*., 2005).

More than 60 % of produced sewage sludge in USA is land applied, and is expected to increase up to 80 % by 2010, while landfill disposal is at 34 % and may decrease to 30 % (USEPA, 1999). In UK sewage sludge land application was estimated to increase from 50 to 66 % and a landfill disposal reduction from 10 to 6 % between 1995 to 2005 (Bowler, 1999).

1.2.2 Advantages of sewage sludge land application

Sewage sludge is an organic material rich in plant nutrients and potentially could enhance soil fertility as a supplier of plant nutrients and improver of soil physical properties. Therefore land application has been considered a better utilization option (Mc Grath *et al*., 1994; Smith, 1996; Peverly, 1996; Snyman *et al*., 1998; Kelly *et al*., 1999; Bowler, 1999; Debosz *et al*., 2002; IWA, 2003; Bengtsson and Tillman, 2004; Van Niekerk *et al*., 2005; Hseu and Huang, 2005).

Based on the advantages of the sewage sludge land application strategy, one could consider it as a solution for both agricultural and environmental problems stated above. Because it can enhance nutrient status of soil and reduce the level of the pollution risk, however, have some disadvantages.

1.2.3 Disadvantages of sewage sludge land application

Though sewage sludge land application strategy has a beneficial effect on soil fertility recovery and the maintenance of a safe environment, an excessive application may cause serious human health and environmental problems, as a result of heavy metal pollution, pathogens and NO_3-N pollution of surface and ground waters (Wortman and Binder, 2002).

Based on the stated disadvantages it is obvious that a comprehensive soil nutrient management plan is decisive to maintain both the agronomical and environmental sustainability of sewage sludge land application (Bastian, 2005). Rulkens (2003), Snyman and van der Waals (2004) reported on the importance of establishing sustainable regulations for sewage sludge use in agriculture. Characterization of sewage sludge and determination of the breakdown and release of nutrients and other elements are important considerations when determining suitable application rates (USEPA, 1994; Navas *et al*., 1997; Wortman and Binder, 2002; Bengtsson and Tillman, 2004).

1.2.4 Sewage sludge use regulations in South Africa

Not all produced sewage sludge are feasible for agricultural use, deciding whether such sewage sludge meet the legal requirements for use is a conjectural process and a great responsibility attributed to several government departments. The Department of Water Affairs and Forestry (DWAF), Department of Environmental Affairs and Tourism (DEAT), Department of Health (DoH) and Department of Agriculture (DoA), join their efforts on maintaining a sustainable utilization of sewage sludge. For them to authorize, sewage sludge must go through the South African waste water sludge classification system.

Sewage sludge classification is based on three classes: the Microbial, the stability and the pollution classes, with three levels each (Table 1.1). Therefore, sewage sludge is tested for several criterions in order to be placed on the respective type. For Microbial class the criteria are faecal coliforms and helminth ova content, for pollution are certain heavy metals and elements considered potentially toxic and for stability the indicator is the vector attraction potential (Snyman and Herselman, 2006).

Table1.1: Sewage sludge classification system

Classes	**Levels**		
Microbial	A	B	C
Stability	1	2	3
Pollution	a	b	c

Sewage sludge of microbial class "A", Stability class "1" and pollutant class "a", is used for land application, on the rate established, while all other classes have same restrictions. However, if it falls to class "B", "2" no matter if pollutants are at level "a" its use is restricted. When, is sludge of microbial class "C", is not allowed for agricultural use (Snyman and Herselman, 2006).

South African guidelines recommend application rates not exceeding crop N requirement to an upper limit of 10 tons of sewage sludge per ha per year, to prevent NO_3-N leaching (Snyman and Herselman, 2006). Differences in the sewage sludge sources and soil types might exert considerable influence on sludge N availability, though not considered.

The relative composition of domestic and industrial waste streams contributes to the final nutrient content of sewage sludge. Sewage sludge stability, which is a function of treatment process, may influence the way sewage sludge release nutrients. This explains the reason why relative composition of sewage sludge from different loads in the same treatment plant differs.

The majority of N in sewage sludge is present in an organic form and has to be converted into inorganic N forms that are available for plants. This conversion process is governed by soil living organisms, therefore soil environmental factors influencing microbial activity, will greatly influence the N mineralization rate.

Understanding the fate and transformations of nitrogen in sewage sludge amended soils is important for effective use of sewage sludge as soil amendment, in order to meet crop demand and at the same time also minimize environmental problems (Serna and Pomares, 1992; Gaines and Gaines, 1994; Smith *et al*., 1998; Waddington, 2003).

Furthermore, research on the release of N from sewage sludge amended soils is necessary to parameterize models in order to predict N and nutrient balance, and gain short, medium and longer term predictive capability on N dynamics Modeling the movement of N in sewage sludge amended soils involves various parameters, such as temperature, moisture regime, quality of sewage sludge and period from application.

1.3 Sample handling strategy for N determination in sewage sludge amended soils

Changes in soil chemical properties ‘nutrient forms and content’ occur as a result of pre-treatment given to soil sample after collection, nitrogen element, is easily transformed within its speciation forms.

Mineralization and nitrification are ongoing processes. Therefore, the handling of biosolid amended soil samples will determine how representative the determined nitrate and ammonium speciation is to what is available in the soil at the time of sampling.

Field validation of mineralization and nitrification rates is essential for accurate prediction and modeling of the environmental fate of nitrogen entering the soil system through biosolids application.

Soil nitrate and ammonium levels are temporarily highly variable as the net result of mineralization, immobilization, leaching, volatilization and denitrification; change with soil water content, soil temperature, quantity and quality of organic inputs (Follett, *et al*., 1987; Stenger *et al*., 1995; Er, *et al*., 2004; Hai-Xing and Sheng-Xiu, 2006).

Inadequate sample handling procedure after sampling may lead to results that are not representative to the site situation. Therefore soil sampling and handling procedures should be consistent and representative.

This dissertation consists of two experiments to investigate: temperature, water potential and sludge stability effect on N mineralization, and the second was to test three sample handling procedures (Direct field extraction, Field dried extraction and Laboratory dried extraction) in a sewage sludge amended soil.

1.4 Objectives of the study

The objectives of the study were to:

i) Determine the net inorganic nitrogen release [(NO_3^- plus NO_2^-) and NH_4^+] as a function of temperature and water potential;

ii) Determine the influence of sewage sludge stabilization on N release;

iii) Determine the rate constant, potentially mineralizable N, and half life time ;

iv) Assess the influence of soil sample handling on the dynamic of NO_3-N and NH_4-N speciation in sewage sludge amended soils.

To fulfill these objectives, a laboratory incubation study was conducted under different environmental conditions in terms of temperature and soil water using a sandy clay loam soil. The soil was amended with sludge, corresponding to 10 t ha^{-1} on a dry mass basis. Three types of sludge of different stability, collected from different wastewater care works plants were used. A sample handling strategy experiment was also conducted, where three different sample handling procedures were tested based on nitrate and ammonium determinations. This dissertation covers four parts as follow: General introduction, Literature review, Incubation experiments and Sample handling strategy experiment.

CHAPTER II: LITERATURE REVIEW

2.1 Introduction

Developing countries are faced with low crop production, caused either by the continued utilization of soils with inherent low soil fertility or soil fertility degradation. Nitrogen and phosphorus are the common plant nutrients limiting the crop production; supply of these nutrients through inorganic fertilizers increases the crop production. However, most farmers do not have financial support to purchase fertilizers (Stoorvogel and Smaling, 1990; Buresh *et al.*, 1997; Folmer *et al.*, 1998; Scoones, 2001; Waddington, 2003). Organic sources are valuable nutrient sources to supplement inorganic fertilizers and an alternative for resource poor farmers to increase crop yield.

Organic sources can encompass any remains of plants, animals, microorganisms, animal excreta and municipal solid wastes. These organic material after being broken down, turns into important sources of plant nutrients and helps to maintain or build up soil organic matter.

In general soil organic matter has a positive effect on the physical, chemical and biological soil properties, such as water retention, aeration, erodibility, cation exchange capacity, nutrient availability and microbe activity. Therefore, soil organic matter is a key component of the soil, "the foundation of a fertile soil". Hence the maintenance of sufficient soil organic matter levels is a prerequisite for sustainable crop yields. For this reasons, research on soil fertility management in developing countries is currently oriented to increase crop production using organic sources with minimal use of inorganic fertilizers (Ward *et al.*, 1987; Buresh *et al.*, 1997; Waddington, 2003; Wolf and Snyder, 2003).

These sources are an economically and environmental viable options, only if well managed. However, the efficient use and management of organic sources requires a good understanding of their nutrient release "mineralization processes".

According to Hseu and Huang (2005) more than 50 % of the total N in sewage sludge is organic, quoting (Sommers, 1977) therefore, it is necessary to determine N mineralization rate and predict N availability.

Mineralization of organic N in sewage sludge amended soils is a complex process mediated by soil organisms that are influenced by several factors such as soil type, pH, temperature, moisture, quality and quantity of applied sewage sludge, (Serna and Pomares, 1992; Sierra *et al*., 2001; Hernandez *et al*., 2002; Wang *et al*., 2003; Zaman and Chang, 2004; Van Niekerk *et al*., 2005; Agehara and Warncke, 2005).

Though mineralization rate is also a function of factors other than climatic ones (temperature and moisture), the obtained models for nitrogen mineralization considered these two as the dominant soil environmental factors. Therefore, they are still empirical models and cannot be reliably applied to a particular soil situation, because they miss factors like soil type (Leiros *et al*., 1999; Van Niekerk *et al*., 2005).

This chapter will focus on nitrogen dynamics in the ecosystem, as well as factors affecting N transformations among different N-forms; the economical and environmental problems of production and disposal of sewage sludge; on the importance of sewage sludge use in agricultural lands, on N mineralization processes occurring in sewage sludge amended soils and also the kinetics involved on these processes.

2.2 Nitrogen dynamics in the ecosystem

Nitrogen is widely distributed in nature and can be found in the atmosphere, the lithosphere, and the hydrosphere. The atmosphere is the main reservoir of nitrogen with about 78 % of gaseous nitrogen (N_2) which is in equilibrium with all fixed forms of N in soil, seawater, and living and nonliving organisms. The distribution of N is given in Table 2.1. Despite the fact that N is the most abundant nutrient in nature, deficiencies in plants occur frequently in non leguminous cropping systems. Organic nitrogen has to be converted into inorganic N (nitrate- NO_3^- and ammonium- NH_4^+ forms) before it could be used by plants.

Table 2.1 Approximate N distribution in the ecosystem (Havlin *et al*., 2005)

N source	Metric tons
Atmosphere	3.9×10^{15}
Sea (both living and non-living)	2.4×10^{13}
Soil (non-living)	1.5×10^{11}
Plants	1.5×10^{10}
Microbes in soil	6.0×10^{9}
Animals (land)	2.0×10^{8}
People	1.0×10^{7}

The N dynamics is governed by interactions between abiotic soil environmental factors such as soil moisture, temperature, oxygen, and biotic components like soil organisms, plants, and by agronomic practices (McGill and Meyers, 1987; Leijder, 1988; Brady and Weil, 2002; Havlin *et al.*, 2005). Understanding the dynamics of different N pools in the ecosystem is an important tool to assess and predict soil N availability (Russell's, 1988; Havlin *et al.*, 2005).

2.2.1 The nitrogen cycle

The conceptual idea of the N cycle date back to 1913 when it was first formulated by Lohnis, and since 1950's diagrams have been drawn to illustrate the pathway of N in the ecosystem (Paul and Clark, 1988). However, its complexity is scientifically challenging (Jarvis, 1996). Cycling of N involve many transformations between inorganic and organic forms (Fig. 2.1).

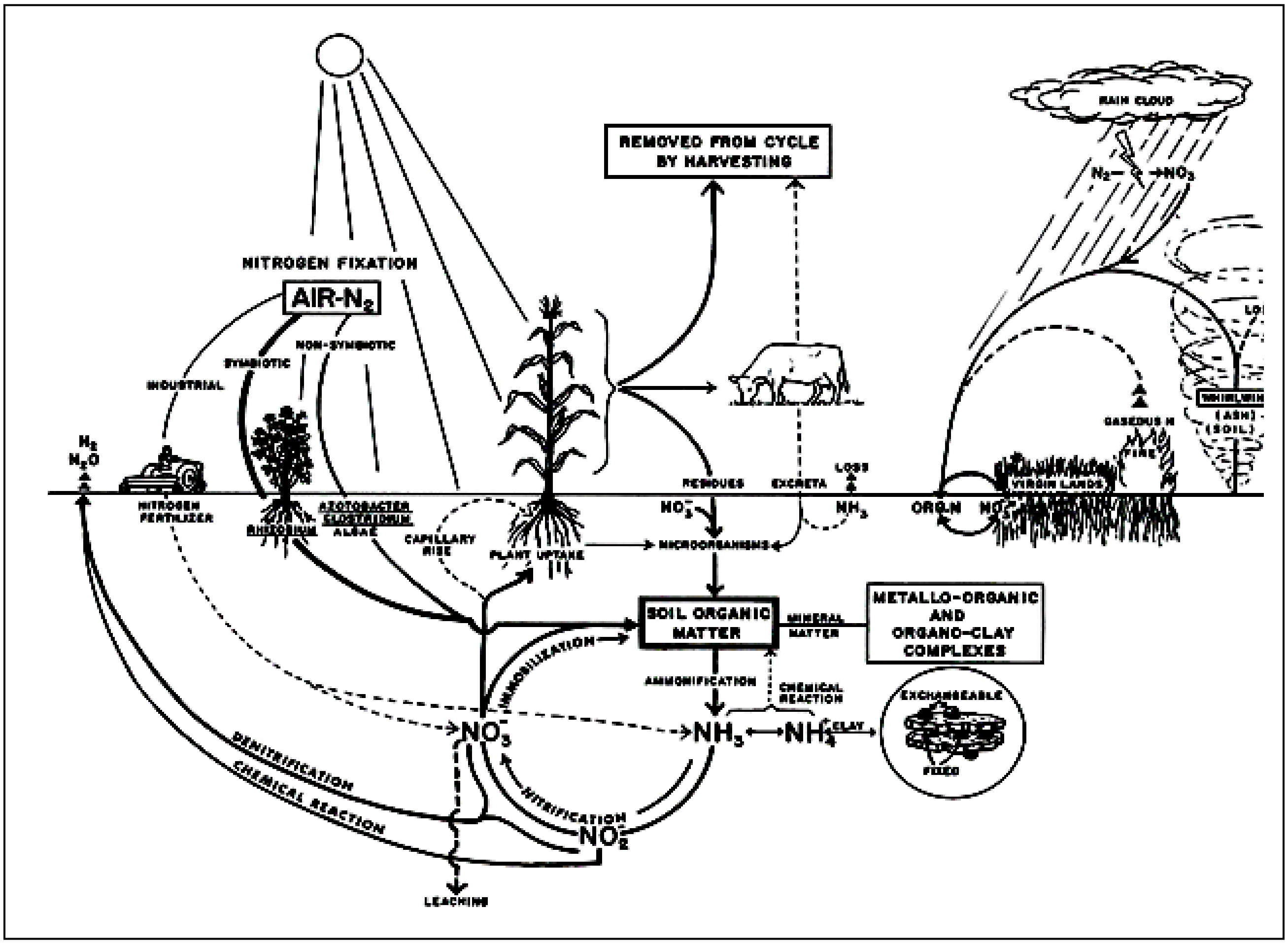

Figure 2.1 Nitrogen cycle in the ecosystem (soil/plant/animal/air) (Stevenson, 1982)

The atmosphere is the primary source of N as shown in the above figure, whereby lightning oxidizes the atmospheric N_2 into NO_3^-- N that is deposited in soils through rain precipitation, fixation through free living bacteria, and symbiotically leaving bacteria, industrially N-fixation and man application of organic and inorganic sources. Organic materials in soils undergo decomposition and accumulate as soil organic matter that contains plant nutrients in organic forms, which in turn can transform into inorganic forms through mineralization.

Inorganic N in the form of NH_4^+-N and NO_3^--N can be taken up by plants, or immobilized by soil microorganisms. Soil microbial population and plants compete for inorganic N forms. Rapidly growing microorganisms can immobilize NH_4^+ and NO_3^-, therefore, depleting temporarily the availability of N to plants. NH_4^+ can also be adsorbed on the edges of clay particles, or fixed in soil clay minerals such as illite and vermiculite; meanwhile NO_3^- can also be lost to the atmosphere through denitrification or leached below the active root zone (Brady and Weil, 2002; Havlin *et al.*, 2005).

2.2.2 Mineralization of organic nitrogen

A significant component of soil total N is in organic forms, and can be converted into inorganic N forms available to plants through mineralization, a biochemical process mediated by microorganisms. The process involves two steps: ammonification and nitrification (Stevenson, 1982; Paul and Clark, 1988; Brady and Weil, 2002; Havlin *et al.*, 2005; Canali and Benedetti, 2005).

1st step: Ammonification process

Firstly the soil organic N compounds undergo an amminification process in which amino–N compounds (R-NH_2) are formed which, in turns, are converted into NH_4^+, in presence of heterotrophic organisms. These organisms are able to operate in both aerobic and anaerobic conditions.

$SOM \rightarrow R - NH_2 + 2H_2O \rightarrow OH^- + R - OH + NH_4^+$, (Brady and Weil, 2002)

2nd step: Nitrification process

The obtained ammonium (NH_4^+-N), in the presence of nitrifying autotrophic bacteria and oxygen (aerobic conditions), is first oxidized into nitrite (NO_2^- -N) in presence of *nitrosomonas* and then to nitrate (NO_3^- -N) through *nitrobacter.*

$NH_4^+ + O_2 \rightarrow 4H^+ + energy + NO_2^- + \frac{1}{2}O_2 \rightarrow energy + NO_3^-$, (Brady and Weil, 2002)

2.2.3 Inorganic nitrogen losses

Not all mineralized N is used by plants and microorganisms, a fraction of it can be lost through volatilization, denitrification and leaching.

2.2.3.1 Volatilization

May occur under alkaline or dry soil conditions, losses can vary from 3 to 50 %; volatilization increase with increasing temperature up to about 45 ^{o}C

$NH_4^+ + OH^- \rightarrow NH_4OH \rightarrow NH_3^{\uparrow} + H_2O$ (Brady and Weil, 2002)

In calcareous soils volatilization is given by the equation.

$$2\,NH_4^+ + CaCO_3 \rightarrow (NH_4)_2CO_3 + Ca$$

$(NH_4)_2CO_3 \rightarrow 2NH_3^{\uparrow} + H_2CO_3 \rightarrow H_2O + CO_2$ (Havlin *et al.*, 2005)

2.2.3.2 Denitrification

Occur under anaerobic conditions, and warm environment. The anaerobic organisms obtain their oxygen from the nitrate and nitrite ions

$2NO_3^- - O_2 \rightarrow 2NO_2^- - O_2 \rightarrow 2NO\uparrow - \tfrac{1}{2}O_2 \rightarrow N_2O\uparrow - \tfrac{1}{2}O_2 \rightarrow N_2\uparrow$ (Brady and Weil, 2002)

2.2.3.3 Leaching

Nitrate ions are very soluble and highly mobile in the soil. Therefore, soil water exceeding the water holding capacity result in excessive water movement causing losses of NO_3^- through runoff and leaching processes (Havlin *et al.*, 2005).

Understanding the gain and loss processes for distinct N-forms, as well as the factors influencing their changes, forms the basis of an efficient management of N in agricultural land. In general, losses can range between 40 to 60 % of applied N, however, under poor management losses may reach 80 % (Leijder, 1988).

2.3 Occurrence and abundance of nitrogen in soils

Generally a high proportion of the total N in surface soils is organic (about 95 %). N content in mineral soils may vary between 0.02 to 0.5 %, while organic soils exhibit values up to 2.5 %. In general soil organic matter (SOM) contains about 5 % of N, therefore, the distribution of N in soils follows the same pattern as SOM distribution. For instance, aridisols are generally poor in both organic matter and organic N, on the other hand histosols and mollisols are rich in organic matter and consequently high in organic N. Andisols are an exception, having higher organic C than any other mineral soil, the reason is the presence of allophane clays that bind organic matter protecting it from being oxidized (Mengel and Kirkby, 1987; Brady and Weil, 2002; Havlin *et al*., 2005).

2.3.1 Forms of nitrogen taken up by plants

Plant roots absorb N in soil solution in the forms of NO_3^- and NH_4^+ ions, the uptake of NO_3^- implies an exudation of HCO_3^- and OH^- from the roots increasing the pH of the rhizosphere. NH_4^+ uptake is accompanied by the release of H^+ from the root into the soil solution resulting in a decreasing of the pH of the rhizosphere. In both cases the effect on pH may influence the availability and uptake of other nutrients. Under field conditions the rate of NH_4^+ uptake is lower compared to NO_3^-, as a result most crops have higher response to NO_3-N applications than to NH_4 -N fertilizers due to high mobility of nitrate and possible fixation of ammonium (Mengel and Kirkby, 1987; Brady and Weil, 2002; Havlin *et al.*, 2005).

2.3.2 Role of nitrogen in plants

Nitrogen is a very important element for plant growth as an integral component of many plant compounds, such as chlorophyll, and proteins. Therefore, N has an important role in the photosynthesis process, carbohydrates utilization within the plant as well as in the transferring of genetic characteristics. N also stimulates the uptake of other plant nutrients, and induces vegetative growth (Stevenson, 1982; Leijder, 1988; Mengel and Kirkby, 1987; Havlin *et al*., 2005).

The nitrogen content in plant varies with plant age and depends on the plant part. The removal of soil N by crops also vary between plant species, being low in root crops 0.5 to 1.0 %, medium in trees and grain crops 1 to 2.5 %, and high in leguminous crops 3 to 5 % (Leijder, 1988).

2.3.3 Oversupply of nitrogen

Excessive N supply decreases the quality of products, because N enhances excessive vegetative growth, poor flowering and seed formation, and retard maturation. Plants may also grow taller and be more susceptible to lodging when exposed to wind and rain. Oversupply of N can also weaken tissue resulting in high susceptibility to pest and fungal diseases, e g. chocolate spot in maize, brown rust in barley, brown leaf spot in rice and *fusarium graminearum* in wheat. Undesirable color and flavor of fruits, lower sugar and vitamin content of certain vegetables and crop roots are also reported (Leijder, 1988; Mengel and Kirkby, 1987; Brady and Weil, 2002; Havlin *et al*., 2005). Another negative effect is that an excess NO_3^- in soils may lead to environmental degradation of groundwater due to leaching and surface water due to runoff (Brady and Weil, 2002). Soil NO_3-N exceeding the permissive contaminant level will negatively affect water quality (Sparks, 2003). Drinking water polluted with NO_3^--N causes diseases in animals and humans such as *methemoglobinemia* or *blue baby syndrome* (Brady and Weil, 2002).

The department of National Health and Population Development in South Africa established 10 mg L^{-1} N in the nitrate form, as the upper standard value for drinking water (Korentajer, 1991); This value is equal to the limit set by the United States regulatory agency for environmental protection (Brady and Weil, 2002; Sparks, 2003).

2.3.4 Deficiency of nitrogen

Soil nitrogen deficiency limits crop productivity as it decreases the production level and quality of products (low protein and high sugar contents), The main symptoms are leaves with pale yellow green colors (*chlorosis*), which is first observed in the older leaves due to translocation of proteins from its chloroplasts to younger leaves. Other symptoms includes die-back from the tip, stunted plants, thin and spindly stems (low shoot-to-root ratio), and quicker maturity than healthy plants (Mengel and Kirkby, 1987; Paul and Clark, 1988; Leijder, 1988; Russell's, 1988; Brady and Weil, 2002; Havlin *et al*., 2005).

2.4 Factors influencing nitrogen mineralization

Mineralization and immobilization processes are mediated by soil organisms, therefore all factors influencing the occurrence and activity of soil organisms will affect N mineralization/ immobilization turnover (NMIT). Environmental factors (temperature and moisture), nature, quality and abundance of organic source, soil type influence mineralization process, as they affect microbial activity (Terry *et al.*, 1981; Mengel and Kirkby, 1987; Paul and Clark, 1988; Russell, 1988; Jarvis *et al.*, 1993; Leiros *et al.*, 1999; Brady and Weil, 2002; De Neve *et al.*, 2004; Er, *et al.*, 2004; Snyman and Van der Waals, 2004; Zaman and Chang, 2004; Havlin *et al.*, 2005).

2.4.1 Soil microbe biomass (SMB)

Soil microbial biomass (SMB) is an important component of soil organic matter (SOM) that regulates transformation and storage of soil nutrients. It forms part of the labile fraction of SOM, and contains 1 to 3 % of total carbon and up to 5 % of the total nitrogen. To understand the nutrient fluxes in natural and agricultural ecosystems, evaluation of the size, diversity and activity of the SMB are necessary (Hortwath and Paul, 1994). Additions of carbon in the form of sugars leads to an increase in SMB activity and consequently a higher N released due to the increased N mineralization (Heal *et al.*, 1997; De Neve *et al.*, 2004).

2.4.2 Soil water content and potential

Soil water content and potential are important factors controlling the microbial activity, and in turn, soil organic carbon and organic N turnover. Soil water influences the mobility of microbial cells in soil while water potential determines the ability of microbes to maintain their activity and survival during periods of water stress. Soil water also affects aeration, and regulates the oxygen supply to microbes (McInnes *et al.*, 1992).

Both low and high soil water content influence the microbial activity negatively. Sierra *et al.* (2001) found nitrifiers more sensitive to changes in water potential, where their activity was inhibited at -1500 kPa. Low soil water content decreases the mobility of soil microbes thus reducing the microbial activity, while high soil moisture creates an anaerobic condition. Therefore, limiting the availability of oxygen to SMB thus, limiting the activity of nitrifying bacteria and favor denitrification process.

It is reasonable to expect that at water potential between –10 to –30 kPa, often used to approximate field capacity, optimal microbial activity can be expected. At this stage the soil water is available for plants and also for microbial growth. Soil microbial activity was reported to be optimum at –50 kPa and decreased as the soil becomes waterlogged (near zero water potential) or more dry (high negative water potentials). While at reduced soil water potential close to –1500 kPa plants suffer from water stress and microbial growth and its activity are depressed, (Mengel and Kirkby, 1987; Paul and Clark, 1988; Leiros *et al*., 1999; Tan, 2000; Havlin *et al*., 2005). Fig. 2.2 shows the influence of soil moisture on the soil microbe activity.

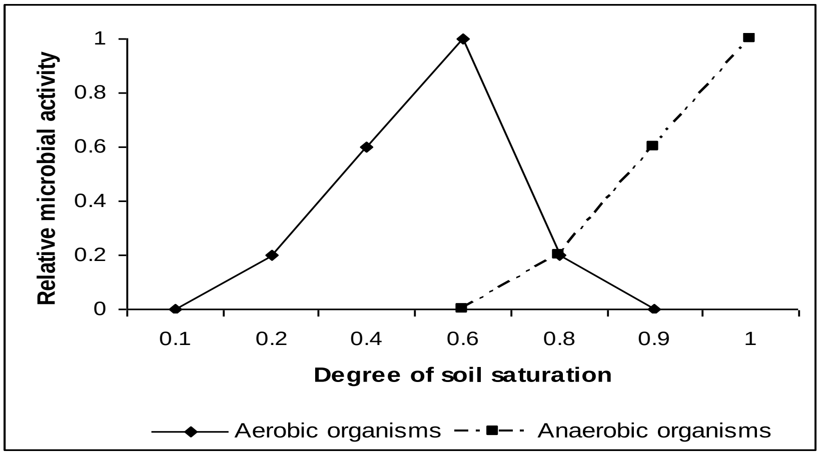

Figure 2.2 Influence of soil moisture on relative microbial activity (Doran and Smith, 1987).

According to Doran and Smith (1987) the activity of aerobic organisms reaches its maximum when 60 % of pore space is filled with water, and is restricted below 20 % and higher than 80 % (equivalent to dry and water-logged conditions). The soil moisture regulates the proportion of nitrifying and denitrifying bacteria’s activity. From Fig. 2.2 it is evident that well-aerated soil favor aerobic nitrifying bacteria and anaerobic conditions enhance the activity of denitrifying bacteria. High salt concentration leads to osmotic stress inhibiting microbial activity. Nitrifying bacteria are very susceptible to salinity (Paul and Clark, 1988; Jarvis *et al*., 1993; Heal *et al*., 1997).

2.4.3 Temperature

Temperature is one of the main environmental factors controlling microbial activity, and therefore the decomposition and mineralization processes. The influence of temperature on nitrogen mineralization can be evaluated through the following equations:

i) Arrhenius equation- which assumes the energy of activation for the process to be constant;

$$N = e^{-Ea/R\left(1/t - 1/T\right)}$$

Where **N** is the rate of nitrogen mineralization at temperature t, T is the optimal incubation temperature, E_a the activation energy expressed in kJ mol^{-1}.

ii) Van't Hoff equation, which assumes the exponential relationship between the rate of the mineralization process and the temperature:

$$N = e^{b(t-T)}; \quad Q_{10} = e^{10b}$$

Where N stands for mineralization rate, b a rate constant, t the temperature of mineralization, T the optimal incubation temperature, and Q_{10} is the temperature coefficient. This coefficient (Q_{10}) is equal to 2 over the range of 5 to 35 ^{o}C, meaning that change in mineralization and nitrification rate is twofold when temperature shifts in 10 ^{o}C.

Temperature increase accelerates the decomposition of organic matter and the mineralization process, up to a certain threshold. High temperature (> 45 ^{o}C) has a negative effect on these processes. The optimum temperature ranges between 25 to 35 ^{o}C, at extreme temperatures such as below 5 ^{o}C and higher than 40 ^{o}C, the microbial activity is depressed or ceases (Mengel and Kirkby, 1987; Paul and Clark, 1988; Brady and Weil, 2002; Havlin *et al.*, 2005). Figure 2.3 shows how microbial activity varies with temperature. Soil microbe biomass activity reaches hundred percent or its maximum within 25 to 35 ^{o}C, and at temperature less or equal 5 ºC and higher than 55 ºC no mineralization occurs, the microbe activity is inhibited. Therefore, high levels of nitrogen released are expected within 25 to 35 ºC. Incubations under 20 ºC and over 40 ºC are expected to produce lower levels of mineralized nitrogen. Sierra *et al.* (2001) reported that at 30 ºC had greater N mineralization, and nitrification increased with temperature. Tajeda *et al.* (2002) found also that N mineralization was higher at 25 ^{o}C than 15 ^{o}C and that increasing temperature boosted mineralization as well as N losses which can exceed 50 %.

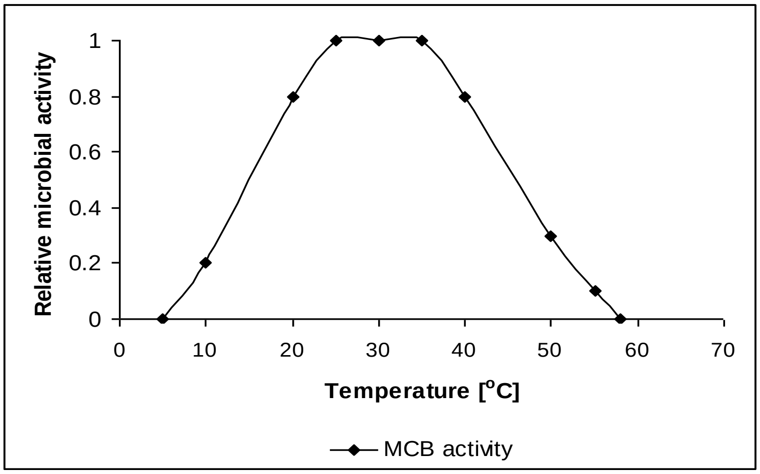

Figure 2.3 Influence of temperature on microbial activity (Doran and Smith, 1987).

2.4.4 Substrate quality

In order to grasp the complexities involving organic substrates it is commonly conceptualize as discrete fractions related to their degradability: a pool of easily decomposable compounds also known as the rapid release pool, a pool of slow release and a third pool of resistant compounds. Besides the environmental soil conditions the rate of N mineralization is also influenced by the quality of organic source and the stability of the organic N compounds present (Smith *et al.*, 1998). Sewage sludge follows the same trend as conversion of its organic N into inorganic N is influenced by its composition and stability. The C:N ratio and also the amount of lignin and polyphenols, exert an important role on the decomposition rate of organic material. At C:N ratio greater than 25 the mineralization will be negatively influenced as immobilization of released N may occur initially. Substrates with C:N ratios less than 20 decompose rapidly. Wolf and Snyder (2003) also reported a C:N of 20 to be the threshold level.

According to De Neve and Hofman (1996) many researchers have tried to quantify the critical C:N ratio for N mineralization, and found it to be 20 for short-term incubations and 30 – 40 for long-term incubations. This dependence of the critical C:N ratio on the incubation period could be explained through the consumption of N by soil organisms which become remineralized after the decay of microbial cells during the incubation.

According to Whitmore and Handayanto (1997) decomposition and mineralization are related. The N mineralization can be expressed as a function of decomposable organic carbon as follows:

N mineralized = C decomposed (1/*z* – *E*/*y*),

Where ***z*** represents the C:N ratio of the added organic material, ***E*** stands for microbiological efficiency factor "representing the fraction of decomposed C that is transformed into SOM", 0.4 is the established value used in APSIM (Agricultural Production Systems Simulation Model) for soil N, and ***y*** the C:N ratio of the recently formed SOM.

Palm and Sanchez (1991) reported that lignin and poliphenols are also determinants of N release from organic sources. Organic materials with considerable high lignin and poliphenol content, and/or high ratio poliphenol:N, cannot readily supply N. The existence of poliphenol-N polymers slow down the decomposition process. However, organic sources with low lignin content and low lignin to N ratio or low poliphenol to N ratio can be used successfully as a source of available N due to the relatively fast decomposition and mineralization rates.

It was found that polyphenolic compounds in the organic source influence NMIT in two ways:

- **i)** Polyphenolic compounds have direct toxicity effect on the SMB;
- **ii)** Polyphenolic compounds have high affinity for amide groups and can bind proteins, preventing N release (Heal *et al.*, 1997; De Neve *et al.*, 2004).

2.4.5 Time

The dynamics of N in soils is governed by mineralization and nitrification processes' changing continuously depending on the environmental conditions at specific time. Since factors controlling soil microbial activity change with time, therefore the length of incubation period would affect the quantity of N released and chemical composition of the soil medium. It was observed that this fact limits the use of mineralization models in predicting the long term N mineralization process (De Neve and Hoffman, 1996; Maly *et al.*, 2002; Benbi and Richter, 2002).

2.4.6 Soil pH

Both microbial diversity and activity are pH dependent. According to Brady and Weil (2002) decomposition and mineralization processes occur rapidly at near neutral pH and optimum moisture and aeration conditions. Under extreme acid conditions decomposition is inhibited. Nitrifying bacteria are more effective under slightly acid to neutral pH (6.6 to 8.0), below pH 6 nitrification rate declines and is negligible below pH 4.5 (Jenkinson, 1981; Terry *et al.*, 1981; Mengel and Kirby, 1987; Paul and Clark, 1988). Sierra *et al.* (2001) also found that in a soil with pH 4.9, an introduction of nitrifiers with fresh sewage sludge had no effect on nitrication rate.

2.4.7 Soil texture

The effect of soil texture is indirect and expressed through soil structure and porosity, thus, regulates the soil water content for a particular water potential. The effect of soil texture manifests as follow:

- **i)** Influences aeration and moisture status;
- **ii)** Affects physical distribution of organic materials and their potential for degradation (Thomsen *et al.*, 1999; Thomsen *et al.*, 2003).

Similarly to Thomsen findings, Jarvis *et al.* (1996) and Hassink *et al.* (1992) concluded that high clay content may decrease mineralization by two mechanisms:

- **i)** Physically confining micro-organisms in small pores making them less active;
- **ii)** Physically protecting non-living SOM from decomposition by surface adsorption on clay minerals.

The majority of mineralization studies have relied on moisture conditions adjusted to water field capacity (WFC) rather than water potential which makes it difficult, if not impossible, to compare mineralization rate across different textured soils, since the soil moisture held at WFC of different textured soils differ (Thomsen *et al.*, 2003).

Hassink *et al.* (1992) found that sieving soils caused a temporary increase in mineralization of carbon and nitrogen, the increase was larger in loam and clayey soils than in sandy soils. This can be attributed to an increase in the contact surface between soil organisms and soil organic materials, which in turn depends on the soil water content. Similarly Stenger *et al.* (1995) found that nitrogen mineralization rates were twice as high in sieved soils compared to undisturbed one.

Hernandez *et al.* (2002) studying N mineralization potential in calcareous soils amended with sewage sludge concluded that the organic N mineralization of sewage sludge was influenced by soil type. Greater N mineralization rate was observed in sandy soils (where ranged from 30 % to 41 % of total N) than clayey soils (where organic N mineralized was about 13 % to 24 %). These results are confronting the Hassink's, (1992) and Stenger *et al.*, (1995) findings.

2.5 Net nitrogen mineralized

Theoretically extractable inorganic N encompasses the three forms of inorganic N (NH_4^+, NO_3^- and NO_2^-) extracted with a 1 molar KCl solution at room temperature. The nitrite form is an intermediate stage of nitrification which in turn is jointly reduced with nitrate during the steam distillation by Keeney and Nelson, (1982). Therefore, in this study the term nitrate is extensively used to designate both NO_3^- and NO_2^-.

2.6 Kinetics of nitrogen mineralization

The kinetic of N mineralization is described using first-order equation:

$N_{min} = N_0\,(1- e^{kt})$, (Stanford and Smith, 1972)

Where N_{min} stands for amount of N mineralized at time t; N_0 is the potential mineralizable N; k is the first-order rate constant and t the incubation time.

Several research on N mineralization were based on the first-order equation (Paul and Clark, 1989; Serna and Pomares, 1992; Smith *et al.*, 1998; Rasiah and Kay, 1998; De Neve *et al.*, 2004; Havlin *et al.*, 2005). Similarly, De Neve and Hofman (1996) used the first-order kinetics to describe N mineralization from organic residues:

$$N_{(t)} = N_A\,(1 - e^{-kt})$$

Where N_t is the net N mineralization at time t, N_A is the part of total residue N that was mineralized, k the rate constant and t the incubation time.

Many researchers have studied different kinetic models to describe N mineralization and found discrepancies within undisturbed and disturbed samples. Some researchers used the single first order model, described by Stanford and Smith, (1972), and concluded that the model described better the N mineralization of undisturbed soils. In disturbed soils samples the double first-order model described by Molina *et al.*, (1980) was appropriate to account for the initial flush of N mineralization or for the existence of two considered pools of organic N, the rapidly (N_1) and slowly (N_2) mineralizable N pools (Dou *et al.*, 1996; Hseu and Huang, 2005; Smith *et al.*, 1998c; Benbi and Richter, 2002).

$N_{min} = N_1\,(1- e^{-k_1 t}) + N_2\,(1- e^{-k_2 t})$, (Molina *et al.*, 1980)

$N_o = N_1 + N_2$

Where N_{min} represents the net mineralized N at time t, N_0 is the potential mineralizable N, estimated from N_1 plus N_2 representing the rapid and slow mineralizable pools respectively, and k_1 and k_2 the specific rate constants of inorganic N accumulation and t the incubation time.

Smith *et al.* (1998c) found that potentially mineralizable N was best estimated as 26 % of total applied N for (N_1) and 42 % of total applied N for (N_2). However Dou *et al.* (1996) found that the goodness of fit of different kinetic models depends on the duration of incubation. For instance, under short incubation time (≤ 15 weeks) the single first-order model was found to provide good fit of data and for long incubation period (> 15 weeks) the double first-order model provided better results.

2.7 Half life time ($t_{1/2}$)

In addition to other parameters for modeling N mineralization the half-life time of the organic substrate is also of great importance in modeling the persistence of the organic substances in soil system. The half-life time for a dynamic system is the time required for the substrate to be reduced by half. For an organic substrate in a soil system it is the time needed to decompose and/or mineralize 50% of the initial amount added to soil system. Half life time are calculated based on the exponential decay models ($Y = Yo\ e^{kt}$), which gives an image of the half life constant independent of the initial valor. Where, Yo is the initial quantity and k the decay or growing rate per unit time (Atkins, 1999; Ansie and Roumen, 2004).

Derivation:

$N_t = N_0\ e^{-kt}$; half life time is when $\boxed{N_t = N_0 / 2}$

$N_0/2 = N_0\ e^{-kt}$;

$½ \ln N_0 = \ln N_0 - kt^{1/2}$ $= - \ln [½N_0/N_0] = kt_{1/2}$; $- \ln ½ = \ln 2$ hence $\ln 2 = kt_{1/2}$; Thus half life time is given by $\boxed{t_{1/2} = \ln 2/k}$

2.8 Sewage sludge or biosolid

Sewage sludge, as commonly called in South Africa, refers to a by-product of the municipal wastewater treatment company. It contains organic matter rich in essential plant nutrients, and in some cases also contains liming agents. Demand for better water quality and strict environmental laws lead to an increase in sewage sludge production, during wastewater treatment. Disposal of sewage sludge is an economic and environmental problem. Sewage sludge used to be disposed through land filling, sea dumping, and incineration. However, the incineration practice has been phased out due to high costs of ash treatment; sea dumping is a threat for aquatic organisms, decrease in availability of land area and the long term environmental problems restricted land filling. Therefore, sludge disposal is becoming a serious challenge in the world (Peverly, 1996; Bowler, 1999; IWA, 2003; Sukkariyah, *et al.*, 2005; Walter *et al.*, 2006).

Municipal sludge, therefore, has a value in agriculture as soil conditioner and supplier of plant nutrients (nitrogen, phosphorus and some micronutrients). The nutrient value of the sewage sludge depends on the source of wastewater and treatment process. For example dewatering of sewage sludge improves the physical aspect of biosolids, however, reduces its NH_4^+ content due to removal of soluble NH_4-N with the liquid phase.

Land application and recycling of sewage sludge in agricultural lands is an option that reduces waste transport costs, prolongs the life span of sanitary landfill and reduces environmental pollution, thus is considered the most sustainable approach for disposing sewage sludge (Kaseva and Gupta, 1996; IWA, 2003; Mendoza *et al.*, 2006). In the UK biosolids applications in agricultural lands has been accepted for more than 40 years (Davis, 1989 as cited in Smith *et al.*, 1998), and in USA more than 60 % of produced sludge is land applied (USEPA, 1994).

Although sewage sludge is a valuable resource it could cause negative environmental impact if used improperly. Excessive applications at rates higher than plant N demand, or applications at the wrong time of the year, may increase pollution risk of surface and ground waters (Kaseva and Gupta, 1996; IWA, 2003; Sukkariyah *et al.*, 2005; Mendoza *et al.*, 2006). Other potential risks of sewage sludge include presence of heavy metals, pathogens and organic contaminants.

Therefore, in some European countries the practice of sewage sludge use in agriculture has been debated quite a lot heading for restriction in farmlands because food products are at risk of contamination which in turn might cause health problems (Mendoza *et al.*, 2006). A better destiny of sewage sludge is recycling on green fields for e.g. on parks, sporting fields, road embankments, golf courses (USEPA, 1994). Another strategy is an on-site use as a source of energy (heat, electricity made from biogas). In South Africa the long term use of sewage sludge in agricultural land still needs to be studied under several field conditions (Snyman and Van der Waals, 2004). Currently composting of sewage sludge is an important strategy for use in farmland for food production (IWA, 2003), as many pollutants are reduced.

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CHAPTER III: SEWAGE SLUDGE STABILITY, TEMPERATURE AND SOIL WATER POTENTIAL EFFECTS ON NET NITROGEN RELEASE

ABSTRACT

To take advantage of sewage sludge as a soil amendment, and to negate negative environmental effects, knowledge of N transformation processes in sewage sludge amended soil is required. The effect of temperature, water potential, and the stability of sewage sludge on sludge-N mineralization and nitrification rates were assessed during a 56-day laboratory incubation study. A sandy clay loam soil was amended with sludge from different wastewater treatment processes. Stable anaerobically digested and paddy dried sewage sludge, collected from Vlakplaas municipal treatment plant, unstable waste activated, partially digested and belt pressed sewage sludge, collected from Olifantsfontein municipal treatment plant, and unstable activated, and anaerobically digested sludge from SASOL a petrochemical industry treatment plant. Sludges were applied at a rate corresponding to 10 tons ha $^{-1}$ on a dry mass basis, and incubated at 10, 25 and 45 ºC, and three levels of water potentials, -10, -100 and -1000 kPa. Treatments including a control were carried out in triplicate. Extractable and exchangeable inorganic N-forms (NH_4^+, NO_3^- plus NO_2^-) were determined at six incubation times 0, 1, 7, 14, 28 and 56 days using the method of Bremner and Keeney (1966), followed by the steam distillation method of Mulvaney (1996). Mineralization rate constant and half life time were estimated based on the single first-order kinetics $N_{(t)} = N_o (1- e^{-kt})$ by Stanford and Smith (1972). A general linear model procedure of the SAS statistical program was used to test for significance of differences between treatments. Soil temperature and water potential interactions as well as sludge stability, significantly influenced mineralization. Net N release increased with incubation time and temperature increase. Nitrification was inhibited at 10 ºC for both unstable sludges, and at 45 ºC for all sludges. However, nitrification was observed at both 10 ºC and 25 ºC for Vlakplaas sludge and only at 25 ºC for Sasol sludge. Nitrification was negligible for Olifantsfontein sludge. On average, net N released was higher for unstable sludges. The high quantities of N released from unstable sludge were not a result of the higher mineralizable N potential, but rather as a result of the higher N content. Therefore they had higher N loading rates. Of the total N added 41 % and 36 % were mineralized from Sasol and Olifantsfontein sludges respectively, and 50 % from Vlakplaas sludge. The Sasol sludge yielded a relatively high rate constant (0.093 week $^{-1}$) and relatively shorter half life time (58 days) at 25 ºC compared to the approximated rate constants of municipal sludges. The rate constant for Vlakplaas was 0.042 week $^{-1}$ (half life = 116 days) and 0.049 week $^{-1}$ (half life = 98 days) for Olifantsfontein.

Sludge-N mineralization rate will vary under uncontrolled field conditions, therefore further field validation of the sludge stability effect on N release is needed to regulate safe sewage sludge agricultural use.

***Key words**: Nitrogen, Sewage sludge, ammonium, nitrate, N mineralization, mineralization rate.*

3.1 Introduction

Sewage sludge is a by-product from wastewater treatment plants, rich in organic matter and plant nutrients, which can be used as a soil amendment to enhance the physical, chemical and biological qualities of soils. Like other organic sources, sewage sludge can have positive influences on the properties of the amended soils, such as increased organic matter, nitrogen content, porosity, soil water holding capacity and biological soil quality (Navas *et al.*, 1997; Lopez-Tercedo *et al.*, 2005).

Sewage sludge contains organic forms of nitrogen, which undergo transformation, releasing inorganic N forms, i.e. ammonium (NH_4^+) and nitrate (NO_3^-) (Er, *et al.*, 2004; Ashok, Paramasivam and Sajwan, 2006). Under favourable soil conditions (adequate microbial biomass, soil water) and favourable temperature for microbial activity the NH_4^+ form is converted rapidly into NO_3^-. The availability of N in soils, as well as in sewage sludge amended soils, depends on the N-forms of the compounds present in the substrate and the rate at which inorganic N is released (Paul and Clark, 1988; Russel, 1988; Havlin, 2005). Therefore, knowledge of the dynamics and transformations of different N pools in the ecosystem is an important tool to assess soil N availability.

N mineralization and nitrification rate constants are important parameters in modelling soil N transformations (Smith *et al.*, 1998 a,c). Predictive capability for N release and potential mineralizable N are essential in providing support for decision makers on land application rate and frequency, as well as helping in establishing irrigation intervals that reduce nitrate (NO_3^-) losses from agricultural lands to surface and ground waters.

Sewage sludge stability is known to influence N release characteristics (Smith *et al.*, 1998 a,b). The rate and extent of NO_3 release in sewage sludge amended soils was found to be dependent on sewage sludge type, soil temperature, soil water and quality as well as quantity of applied sewage sludge, (Terry *et al.*, 1981; Paul and Clark, 1988; Serna and Pomares, 1992; Hernandez *et al.*, 2002; Wang *et al.*, 2003; De Neve *et al.*, 2004; Zaman and Chang, 2004; Synman and Van der Waals, 2004; Agehara and Warncke, 2005; Van Niekerk *et al.*, 2005).

Several researchers have conducted incubation studies at field capacity, which is generally accepted as the optimum matrix potential for microbial activity (Thomsen *et al.*, 1999; Thomsen *et al.*, 2003).

Soils are of different textural classes, adjusting soil water to field capacity will not give a comparable situation like when using the concept of soil water potential, which may give an advantage to interpolate the N mineralization rate for different textured soils.

In this study, soil water potential and its interaction with temperature were of concern, because one of the objectives of the study was to find N mineralization and nitrification rates in sewage sludge amended soil that can be used in modeling the fate of N under different soil types and conditions.

A 56- day laboratory incubation study was conducted, as a factorial experiment with temperature as main factor and water potential secondary factor. Soil was amended with sewage sludge of different stability and incubated in a temperature controlled chamber at 10 ºC; in an incubation room at 25 ± 2 ºC and in a temperature controlled chamber at 45 ºC. Samples were extracted with a 1 molar potassium chloride solution and distilled with a micro-Kjeldahl system NH_4^+ and $[NO_3^- + NO_2^-]$ were then determined through titration with a 0.01*M* hydrochloric acid solution (HCl).

According to Doran and Smith (1987) microbial activity is maximal in a temperature range between 25 – 35 ºC. Therefore, high levels of nitrogen release were expected for treatment combination of 25 ºC and -10 kPa. At temperature below 20 ºC and over 40 ºC, lower nitrogen release rates were expected.

Sludge stability is expected to affect the N release rate, estimating higher values for unstable sludge, as the fast release pool of the stable sludge has been partly gone mineralization during the stabilization process in paddy drier beds.

3.2 Materials and methods

A laboratory incubation experiment was conducted at the Soil Science Laboratory of the University of Pretoria, using a red sandy clay loam soil collected from the Hatfield Experimental Farm (University of Pretoria located at 25° 45' S and 28° 16' E).

3.2.1 Materials

i) Sources of sludges

The sludges were collected from three waste-water treatment plants (WWTP), two are branches of the East Rand Water Care Company (ERWAT), a major waste-water care company in South Africa located near Kempton Park Pretoria, and the third was from a petrochemical company (SASOL) located near Secunda. Sasol sludge is not destined for agricultural use, however, it was include to compare the N release from a sludge that originated from the petrochemical industrial with the N release from municipal sludge.

i_1) Vlakplaas WWTP- was a mixture of domestic and industrial wastewater sewage sludge that was anaerobically digested and paddy dried. This stable sewage sludge had an initial solid content of 50 %, and contained 1.93 % N.

i_2) Olifantsfontein WWTP- was domestic wastewater sewage sludge that was activated, partially digested and belt pressed. The sewage sludge collected had an initial solids content of 18 %, and 5.33 % N on a dry mass basis.

i_3) SASOL WWTP- was a petrochemical wastewater sludge that was activated, anaerobically digested. It was an unstable sludge, containing 9 % solids and had an N content of 7.91 %.

3.2.2 Methods

i) Sludge characterization

Sludge samples were sent to the Agricultural Research Council (ARC) - Institute for Soil Climate and Water for analysis of some selected properties. The pH and inorganic N forms were analysed at the Soil Science Laboratory of the University of Pretoria. Table 3.1 shows some chemical characteristics.

i- Total P and exchangeable bases – Acid-mixture digestion followed by colourimetric determinations

ii- Total N determination - Kjeldahl digestion procedure

iii- Total C was determined by means of Walkley and Black method

iv- pH was determined potentiometrically, in a 1:2.5 sludge:water suspension

v- Inorganic N forms (extractable and exchangeable NH_4^+, NO_3^- plus NO_2^-), were determined by the Bremner and Keeney (1966) and Mulvaney (1996) methods

Table 3.1 Some chemical characteristics of the sludges used

Parameters analysed	**Source of sludges**		
	Vlakplaas	**Olifantsfontein**	**SASOL**
pH	6.3	6.7	5.8
Total N (%)	1.93	5.33	7.91
Total C (%)	11.6	29.9	38.9
C : N	6.0	5.6	4.9
Total P (%)	2.43	3.97	0.64
Ca (%)	1.58	3.32	1.07
K (%)	0.11	0.45	0.35
Na (%)	0.14	0.13	0.27
Mg (%)	0.17	0.57	0.35
NH_4 (%)	0.66	0.12	0.47
NO_3 (%)	0.24	0.01	0.01
Org N (%)*	1.03	5.2	7.43

**Org N calculated by difference: % Total N – Inorg N % (NH_4^+ + NO_3^-)*

Although unstable Sasol and Olifantsfontein sludges contained more total N, their initially inorganic N content was lower compared to the anaerobically digested and paddy dried Vlakplaas sludge. The lower values of inorganic N fraction of total N in unstable sludges is the consequence of limited mineralization that have taken place, and the high solubility of ammonium and nitrate in water and its loss during the dewatering stage. In the case of Vlakplaas, relatively high initial inorganic N was observed as more time had passed for organic N gets mineralized during the drying process.

The obtained values in percentage were then converted into mg kg^{-1}, multiplying by 1000/100 to bring them to grams per kg and then multiplied by 1000 to express in mg per kg; i.e. total N (1.93 *(1000/100)) *1000 = 19300

The principle of stechiometry shows that 1mol of either NH_4 or NO_3 gives 1 mol of N. Therefore, for convenience results were expressed in mmol per kg, as the molar basis makes easy and confident comparison among N specimens. Table 3.2 shows the distribution of total inorganic and organic N forms expressed in [mmol kg $^{-1}$] of the sludge samples.

Table 3.2 Sludge N composition

Source of sludges	mmol kg $^{-1}$			
	Total N	**NH_4^+**	**NO_3^-**	**Org N**
Vlakplaas*	1379	367	38.7	736
Olifantsfontein**	3807	66.7	1.6	3714
Sasol**	5650	261	1.6	5307

**stable sewage sludge, ** unstable sludges*

Theoretically, the unstable sludges with high potentially mineralizable N, (98 - 94 %) of total N in organic form (Table 3.3) and with a C:N ratio less than 10 (4.9-5.6) indicating that their organic compounds are easily decomposable (Table 3.1), and expected to have higher N release than the Vlakplaas sludge with 53 % of total N in organic form and a C/N ratio of 6.0. According to De Neve and Hofman (1996), Wolf and Snyder (2003) 20 is the critical C:N ratio for N mineralization for short-term incubations. Meaning that narrow ratio <20 would theoretically allow fast N release and a wide ratio >20 retard it.

Table 3.3 The percentage distribution of N-forms in the sludges.

Source of sludge	NH_4^+	NO_3^-	Org N
		%	
Vlakplaas*	26.6	28.0	53.4
Olifantsfontein**	1.75	0	97.6
Sasol**	4.62	0	93.9

**stable sewage sludge, ** unstable sludges*

ii) Soil characterization

The soil sample was air-dried and passed through a 2 mm mesh sieve, then analyzed for selected soil physico-chemical properties shown in Table 3.4, using the following methods:

i. Particle-size analysis was done using the hydrometer method, (Gee and Bauder, 1986) modified from Day (1965), which consists of measuring the density of a soil suspension based on settling speed of soil particles;

ii. Water retention curve, was determined using the pressure plate extraction method (Dane and Hopmans, 1986);

iii. Soil bulk density ρ_b is the mass per unit volume of soil, and was determined based on the (Grossman and Reinsch, 2002) method;

iv. EC and pH were determined potentiometrically, in a 1:2.5 soil : water suspension (The Non – Affiliated Soil Analysis Work Committee, 1990);

v. Available P was determined using the Bray I method (Bray and Kurtz, 1945) (The Non-Affiliated Soil Analysis Work Committee, 1990);

vi. Exchangeable bases, with the CH_3 $COONH_4$ - pH_7 extraction method, Ca, Mg, Na and K were determined with Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP – AES) (The Non – Affiliated Soil Analysis Work Committee, 1990);

vii. Organic carbon (OC) was determined using the Walkley and Black method (The Non – Affiliated Soil Analysis Work Committee, 1990);

viii. Total N was determined by means of Kjeldahl digestion followed by colourimetric determination (Stevenson, 1996);

ix. Inorganic N forms (extractable and exchangeable NH_4^+, NO_3^- and NO_2^-), were determined by the Bremner and Keeney (1966) method, which consisted of a 1*M* KCl extraction followed by micro Kjeldahl steam distillation and titration with 0.01*M* HCl, (Mulvaney, 1996) (The Non – Affiliated Soil Analysis Work Committee, 1990).

Table 3.4 Selected soil physical and chemical properties

Parameters analysed	**Values**	**Parameters analysed**	**Values**
pH	5.80	P (mg kg^{-1})	34.4
EC (mS m^{-1})	0.12	Ca (mg kg^{-1})	940
Clay (%)	30.0	Mg (mg kg^{-1})	270
Silt (%)	19.2	Na (mg kg^{-1})	8.00
Sand (%)	50.8	K (mg kg^{-1})	75.0
Total N (mg kg^{-1})	300	NH_4 (mg kg^{-1})	8.64
Org C (%)	0.85	NO_3 (mg kg^{-1})	52.3

3.2.3 Treatments

Sludge amended soils were incubated at three levels of temperature and three water potentials for a particular temperature, a schematic representations of treatments is given in Figure 3.1

Treatment selection was based on the established upper limits for sewage sludge land application in South Africa (Snyman and Herselman, 2006), and also on the environmental soil conditions with marked influence in microbial activities, thus influencing nitrogen release (Doran and Smith, 1987; Paul and Clark, 1988; Zaman and Chang, 2004; Leiros *et al.*, 1999; Tan, 2000; Havlin, *et al.*, 2005). Samples were extracted and tested at six different incubation times (0, 1, 7, 14, 28 and 56 days). Treatments were replicated three times. A control treatment was included for each temperature and water potential combination to enable calculation of released N from the sludge. For each sludge 273 samples were analysed for both nitrate and ammonium determination, therefore, in total 719 samples were incubated. Statistical analysis was done using a general linear model procedure of SAS statistical program, considering the incubation as a two factorial experiment.

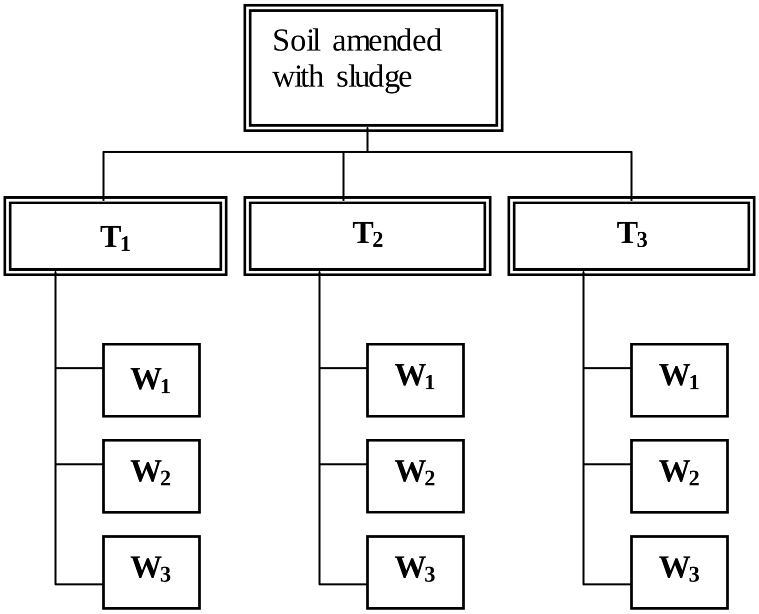

Figure 3.1 Schematic representation of treatments

Where T stands for temperature: $T_1 = 10\ ^{o}C$, $T_2 = 25\ ^{o}C$, $T_3 = 45\ ^{o}C$ and W stands for water potential: $W_1 = -10$ kPa, $W_2 = -100$ kPa, $W_3 = -1000$ kPa. Samples were extracted after 0, 1, 7, 14, 28 and 56 days of incubation.

3.2.4 Procedure

3.2.4.1 Establishing water quantities corresponding to selected water potentials

The quantity of water corresponding to a particular water potential was established gravimetrically based on the water retention curve determination, using the ceramic pressure plate extractor to obtain the relationship between the water content held in soil (θm) and matric potential (ψ_m) (Dane and Hopmans, 1986). Rings were placed on the ceramic plate filled with soil, sufficient water was added to the plates to ensure conditions to approach saturation and left for 24. Afterwards the ceramic plate was taken to the extractor and adjusted to specific pressure left the time needed until no more water was extracted. The plate was then taken out and the content of the rings placed in containers of a know mass and weighed to obtain the wet sample mass (M_{ws}). The samples were then oven dried for 24 hours at 105 °C to obtain the mass of the soil (M_{ds}). The percentage gravimetric water content was obtained based on equation 3 (referred below, under calculations step 4) , and was found to be 17.5, 12 and 10 % corresponding to water potentials of -10, -100 and -1000 kPa respectively.

3.2.4.2 Incubation

The amount of sludge added was equivalent to 10 ton ha^{-1} on a dry mass basis (0.31g, 0.23g and 0.22g) for stable Vlakplaas, unstable Olifantsfontein and Sasol sludges respectively, were added and thoroughly mixed with 50 g of soil pre-incubated, for seven days, at room temperature (~25°C) and moistened with 50% of water corresponding to water potential of -10 kPa (4.25g), to stimulate microbial activity. After mixing the amended soil was weighed and while still on the scale water was added corresponding to 8.5g, 6.0g, and 5.0g to adjust water potentials equivalent to -10, -100, and -1000 kPa respectively. The amended soil was then incubated at selected temperature for 56 days. An incubation chamber with temperature control was used to maintain the temperature of the low temperature treatment at 10 ºC. The 25 ºC treatment was done in an incubation room, while the high temperature treatment was subjected to a constant temperature of 45 ºC in a incubation chamber

Water potential and aeration were monitored as described below. After each incubation time (0, 1, 7, 14, 28 and 56 days), samples were extracted with a 1*M* KCl solution for extractable and exchangeable inorganic N determination (Bremner and Keeney, 1966).

3.2.4.3 Monitoring water potential and aeration

Samples incubated at 10 ºC and 25 ºC were opened and aerated using a small computer cooling fan for approximately 1 minute, every two days to ensure the aerobic condition needed for nitrification. At the same time samples were weighed and water added to compensate for evaporation, in order o maintain conditions as close as possible to the water potential. This was again performed daily for the higher temperature treatment, however, it proved to be difficult to maintain the various water potentials at the high temperature treatment because the samples appeared to dry out quickly. It is more likely that conditions of alternating wetting and drying were simulated for the 45 ºC treatment rather than conditions of near water content.

3.2.5 Calculations

3.2.5.1 Mass of sludge used to amend the soil

The guidelines for sewage sludge application in South Africa established the upper limit application rate of 10 ton per ha per year. Therefore, to get the mass of sludge to be added to 50 g of soil used for incubation, the following calculations were made:

1st step – Soil bulk density (ρ_b), which is the mass of soil per unit volume, was estimated dividing a mass of oven-dried soil (m_s), by the volume occupied by that mass of soil (V_t)

$\rho_b = m_s/V_t$ *(equation 1)*

For a volume of 10 cm^3 the corresponding mass was 11.9 g, therefore the ρ_b was 1.19 g cm^{-3}

2nd step – Estimation of a mass of soil (M) corresponding to the area of 1 hectare, assuming a plough layer depth of 20 cm, and the previously estimated bulk density of 1.19 g cm^{-3}.

$M = V \times \rho_b = A \times d \times \rho_b$ *(equation 2)*

$M = 10\ 000\ m^2 \times 0.20\ m \times 1.19 \times 10^3\ kg\ m^{-3} = 2.38 \times 10^6\ kg$

Where A is the area in m^2, and d is the ploughing depth

3rd step – The mass of sludge needed to apply to 50 g of soil (M_s) is given by:

$M_s = 1 \times 10^4\ kg \times (5 \times 10^{-2}\ kg / 2.38 \times 10^6\ kg) = 2.1 \times 10^{-4}\ kg$

To achieve an equivalent sludge loading rate of 10 ton ha^{-1}, in dry mass basis, 0.21 g of dried sludge was required to amend the 50 g of soil.

Considering that the (M_s) refers to the dry mass basis of sludge and since the collected samples were not completely dry as oven drying sludge is not recommended (N loss may occur), therefore a moisture correction factor was required to obtain the air dried sludge mass to be added (M_{add}).

4th step – To obtain the moisture correction factor it was necessary to determine the moisture content. The thermo-gravimetric method based on convective oven drying at 105 °C (Topp and Ferré, 2002) was used to assess the water content for air dried stable sludge and for the unstable sludges previously dried at 70 °C. Percentage of water was given by the equation:

$$\% \text{ water} = [(m_{bod} - m_{od}) / m_{bod}] \times 100 \qquad \textit{(equation 3)}$$

Where, m_{bod} stands for mass of sludge before oven drying and m_{od} is the oven dried mass of sludge.

Taking the air dried stable sludge as an example, the percentage moisture was 32.1 % based on values presented in Table 3.5.

Table 3.5 Stable Vlakplaas sludge moisture content

Mass	**Replicates mass [g]**			**Mean**	**% moisture**
$\mathbf{m}_{sbod}$	5.00	5.09	5.03	5.04	
$\mathbf{m}_{sod}$	3.36	3.39	3.50	3.42	32.1

$$\% \text{ moisture} = (5.04 - 3.42)/5.04 \times 100 = 32.1\ \%$$

The stable sewage sludge had a moisture content of 32.1 % meaning that 67.9 % of the wet sample was dry sludge. Therefore the moisture correction factor (f) was 100/67.9 = 1.47

Finally the mass of air dried sludge to be added (M_{add}) was given by the following equation:

$$M_{add} = M_{Ss} \times f \qquad \textit{(equation 4)}$$

$$M_{add} = 0.21 \times 1.47 = 0.31 \text{ g}$$

3.2.5.2 Extractable and exchangeable NH_4^+ and NO_3^- plus NO_2^- determinations

The NH_4^+ and $NO_3^- + NO_2^-$ contents in sludge amended soils were determined after each incubation period using Bremner and Keeney (1966) for extraction and Mulvaney's (1996) method for distillation, as described in Sparks (1996). An aliquot of 50 ml from the 100 mL extract was mixed with 20 mL of a 12.5 *M* NaOH solution, which creates conditions for NH_4^+ conversion into NH_3, by means of pH increase to above 9.2.

$$NH_4^+ + OH^- \longrightarrow NH_4OH \longrightarrow NH_3 + H_2O \qquad \textit{(equation 5)}$$

The NH_3 formed volatilized during distillation and was collected in a mixture of boric acid and methyl red plus methyl blue indicators which change colour at a specific pH, forming a green coloured complex (alkaline pH) and purple to slight rose at acid pH

$$\underset{\text{(Purple) pH < 4.4}}{NH_3 + H_3BO_3 / \text{indicator}} \longrightarrow \underset{\text{(Green) pH > 6.2}}{NH_4H_2BO_3} \qquad \textit{(equation 6)}$$

The collected distilled solution was titrated with a 0.01 *M* HCl solution, and the volume recorded was used for calculations, to obtain the amount of NH_4^+ in the sludge amended soil, based on the neutralization principles.

$$\underset{\text{(Green)}}{NH_4H_2BO_3^- + HCl} \longrightarrow \underset{\text{(Purple)}}{NH_4Cl + H_3BO_3} \qquad \textit{(equation 7)}$$

A reducing agent, Devarda's alloy powder, was added to remaining extract in the distil tube after cooling, to convert $NO_3^- + NO_2^-$ to NH_4^+, which in turn, was taken back for distillation to convert NH_4^+ into NH_3 in presence of the alkali in excess (*see equation 5*). The process was repeated the same way as described above. The volume of acid used was recorded for calculations, to obtain the amount of NH_4^+ equivalent to the amount of $NO_3^- + NO_2^-$ in the sludge amended soil, because it is an equimolar displacement reaction. Both NH_4^+ and ($NO_3^- + NO_2^-$) amounts were obtained using the following equation:

NH_4^+ [mmol kg^{-1}] = (V_{acid} x C_{acid})/ 1000 x ($V_{extract}/v_{aliquot}$) x 1000/m $_{(amended\ soil)}$ x 1000 *(equation 8)*

Where $V_{extract}$ is the volume of extracting solution, $v_{aliquot}$ is the aliquot taken for digestion, C_{acid} is the concentration of the acid, V_{acid} is the volume of the acid used in titration for complete displacement of ammonium and $m_{(amended\ soil)}$ the mass of the soil plus sludge added. The first 1000 is for volume conversion from L to ml, and the second 1000 for mass conversion from g to kg, and the third 1000 is for converting mol to mmol.

3.2.5.3 Net N release from the sludge

The term net N release in this study encompasses all forms of inorganic N released from the sludge during incubation processes, plus the initial inorganic N content in the sludge, which are measured in a 1 *M* KCl extraction solution. The individual values of extractable and exchangeable inorganic N forms (NH_4^+ and NO_3^- plus NO_2^-,) were calculated based on *equation 8*. Therefore, net N release was calculated as the difference between extractable and exchangeable NH_4^+ and NO_3^- plus NO_2^- obtained in sludge amended soil and in the soil without any sludge (control) *equation 9*.

Net inorg N released = sludge N amended soil – control N released *(equation 9)*

Net inorganic N released = $[NH_4^+ + (NO_3^- + NO_2^-)]_{amended\ soil} - [NH_4^+ + (NO_3^- + NO_2^-)]_{control}$

3.2.5.4 Potentially available N

This is a very important parameter in soil fertility studies for efficient use of N and for good environmental management. The potentially available N from sludge amended soil expressed in percentage can easily be calculated as follows:

Potentially available N = (Net inorganic N released) / Tot N added) *100 (*equation 10)*

Where **Potentially available N** is the inorganic N, **Net inorganic N released** is the initial inorganic N added through sludge application plus net N mineralized during incubation, and **Tot N added** is the amount of total N present in the added sewage sludge.

3.2.5.5 Organic N mineralized or potential mineralizable N

Part of organic N was mineralized during incubation period, and was calculated by difference between total Org N and organic N present at time t. The potential mineralizable N was expressed in percentage basis and was calculated using *equation 11*. However, potential organic N was underestimated as a result of overestimation of Org N_t (having other inorganic N-forms, which were not captured).

Org N mineralized = Total Org N - Org N_t

Potential mineralized N = Org N mineralized/ Total org N added)]* 100 (*equation 11)*

3.2.5.6 Partial N mass balance

An assessment of the N mass balance was important for this study, because it gives an insight into the fate of the different N-forms during the incubation process. Sludge N was reported on a % dry mass basis (Table 3.1), and therefore, it had to be converted into [mg kg^{-1}] or [mmol kg^{-1}]. **For convenience it is expressed in mmol per kg, as the molar basis makes easy and confident comparison among N specimens**. The mass balance linking different N-forms in sludge amended soils is presented in Table 3.14 for stable sludge, Table 3.15 for unstable Olifantsfontein and Table 3.16 for unstable Sasol sludge.

An example for calculations is shown below, taking the stable Vlakplaas sludge, which contained 0.66 % NH_4^+ and 0.24 % NO_3^- + NO_2^-, in dry mass basis.

i) Amount of NH_4^+ contained in the mass of sludge used for incubation

Based on the percentage NH_4^+ in the stable sludge (0.66 %), the amount of NH_4^+ in the mass of sludge used for incubation was:

NH_4^+ = ((0.66 x 0.21g)/ 100) x 1000 = 1.386 mg / 18 = 0.08 mmol

Where, multiplying with a 1000 was to convert grams to milligrams, and to express the amount of NH_4^+ in mmol results should be divided by the molar mass of NH_4 (1mmol is equal to 18 mg).

ii) Amount of NO_3^- contained in the mass of sludge used for incubation

From Table 3.1, the NO_3^- in the stable sludge was (0.24 %), therefore, the amount of NO_3^- in the mass of sludge used for incubation was:

NO_3^- = (0.24 x 0.21g / 100) x 1000 = 0.504 mg /62 = 0.008 mmol

Where, 1000 is to convert grams into milligrams, and to express the amount of NO_3^- in mmol results should be divided by the molar mass of NO_3

For the other N-forms (Tot N and Org N), calculations were based on the same principle used in **i)** and **ii)** using the molar mass of N (14). However, results from these calculations, shown in Table 3.6, correspond to the N added to 50 g of soil. Therefore, conversion to a kg of soil was needed, to ensure uniformity of units with those of the net N, NO_3^- and NH_4^+ released during incubation period.

Table 3.6 Sludge N-forms contained in 50g of soil amended

Sludge source	Mass of sludge added (g)	(mmol / 50 g soil)			
		NH_4^+	NO_3^-	Org- N	Tot N
Vlakplaas	0.21*	0.08	0.01	0.15	0.29
Olifantsfontein	0.21*	0.01	0.00	0.78	0.80
Sasol	0.21*	0.05	0.00	1.11	1.19

***** *Dry mass basis*

iii) Amounts of sludge N- forms contained in a kg of soil amended

The amounts of sludge N-forms present in 1kg of soil amended were deduced from Table 3.6 multiplying by a factor of 20 coming from 1000g/50g, and are presented in Table 3.7.

Note: Considering that the 10t per ha are applied to a total mass of soil corresponding to a volume of soil given by an area of 1ha and a ploughing depth of 0.20 m, therefore the amount of sludge-N forms should be referred to a 1kg of soil, as shown in Table 3.7.

Table 3.7 Equivalent amounts of N-forms contained in 1kg of soil amended

Sludge source	**mmol kg^{-1} soil**			
	Tot N	**NH_4^+**	**NO_3^-**	**Org- N**
Vlakplaas	5.80	1.60	0.20	3.00
Olifantsfontein	16.0	0.20	0.00	15.6
Sasol	23.8	1.00	0.00	22.2

These amounts initially present in the sludge amended soil before incubation took place, were jointly used with the calculated amounts of net N released during incubation period to obtain the partial N mass balance after 56 day incubation.

3.2.5.7 Mineralization rate constant

Mineralization rate constant and half life time are important parameters required in modelling for predicting N fate in soil systems that are obtained from N mineralization kinetics.

Based on literature it was also assumed that first order kinetics model by Stanford and Smith, (1972) will be the best approach to determine rate constants for N mineralization (Benbi and Richter, 2002; De Neve *et al.*, 2004). Where data obtained from the incubation study was used to draw a graph based on natural logarithm (ln N/N_o) versus incubation time, and the slope gives an estimated mineralization rate constant (k).

$$N_{(t)} = N_o (1- e^{-kt}) \qquad \textit{(equation 12)}$$

Where $N_{(t)}$ is the net N mineralized at time t; N_o the potentially mineralizable N; k is the mineralization rate constant and **t** the incubation time.

Derivation of equation 12

Nitrogen at instant t (N_t) was modeled assuming that the rate of mineralization was constant (k)

i) $dN_{(t)}/dt = k$, proportional to the remaining sludge mineralizable N, which is the difference between potentially mineralizable N (N_o) and the cumulative already mineralized N at time t ($N_{(t)}$)

ii) $dN_{(t)}/dt = k (N_o - N_{(t)})$, not accounting for the initial N or assuming that N content is zero at the beginning of incubation the solution for equation i) is given by $N_{(t)} = kt$. Therefore the solution for equation ii) become $N_{(t)} = N_o (1-e^{-kt})$

Derivation of k from equation 12

The rate constant is calculated as follows, or by plotting a ln N/N_o graph versus time the slope represents k:

$N_{(t)} = N_o (1- e^{-kt})$

$N_t/N_o = 1 - e^{-kt}$

$e^{-kt} = 1- N_t/ N_o$

$- kt = \ln(1 - N_t/N_o)$

$-kt = \ln (1) - \ln (N_t/N_o)$; $\ln (1) = 0$

$-kt = - \ln (N_t/N_o)$; $k = \ln (N_t/N_o)/ t$ *equation 13*

Considering the fact that the first flush of N mineralization on disturbed samples, during the initial two weeks of incubation, is an experimental artefact which results from a drying and rewetting of soil samples. Therefore, the inorganic N measured is not part of true potentially mineralizable N (No) of the substrate and should be modelled separately through the double first order model, by (Molina *et al.*, 1980) to account for the initial flush of N mineralization.

$N_{min} = N_1 (1- e^{-k_1 t}) + N_2 (1- e^{-k_2 t})$ *(equation 14)*

N_1 represents the fast cycling pool of mineralizable N and k_1 the rate constant for this pool; N_2 represents the slow mineralizable N pool and k_2 the corresponding rate constant.

Theoretically it is assumed that these pools are of definite sizes that should not change with environmental conditions or with procedures used to fit the models to data (Cabrera *et al.*, 2005). Based on Smith *et al.* (1998c) findings potentially mineralizable N was also estimated at 26 % of total applied N for the fast cycling pool (N_1) and 42 % of total applied N for the slow release pool (N_2).

3.2.5.8 Half life time

The half-life time for a given exponential decay process indicates how long it would take for 50% of initial amount added to decompose: $Y_t = Y_0 / 2$

The value of t that satisfies the above equation is the half life time and is given by:

$t_{1/2} = \ln (2)/k$ *equation 15*

3.3 Results and discussion

The amounts of NH_4^+, NO_3^- and net N release during incubation are reflected in Figures 3.3, 3.4 and 3.5, illustrating how N mineralization was affected by incubation time, temperature, water potential and sludge stability. Talking on net N release one should expect the graphs of net inorganic N release starting from zero at day zero (as initial stage of the incubation), however, considering the aspect of sludge nitrogen availability for crops, they start from a value representing the inorganic N already present in the sludge.

3.3.1 Net inorganic N released after a 56-day incubation

Figure 3.2 (a) reflects net N mineralized and 3.2 (b) net N released, that is N liberated as a result of the mineralization of Organic N as well as inorganic N released from the sludge. As discussed previously net N mineralized is important in modelling N mineralization, however, net N release is also important from both a soil fertility and environment management point of view, because sludge can contain appreciable amounts of inorganic N depending on the stabilisation process it underwent.

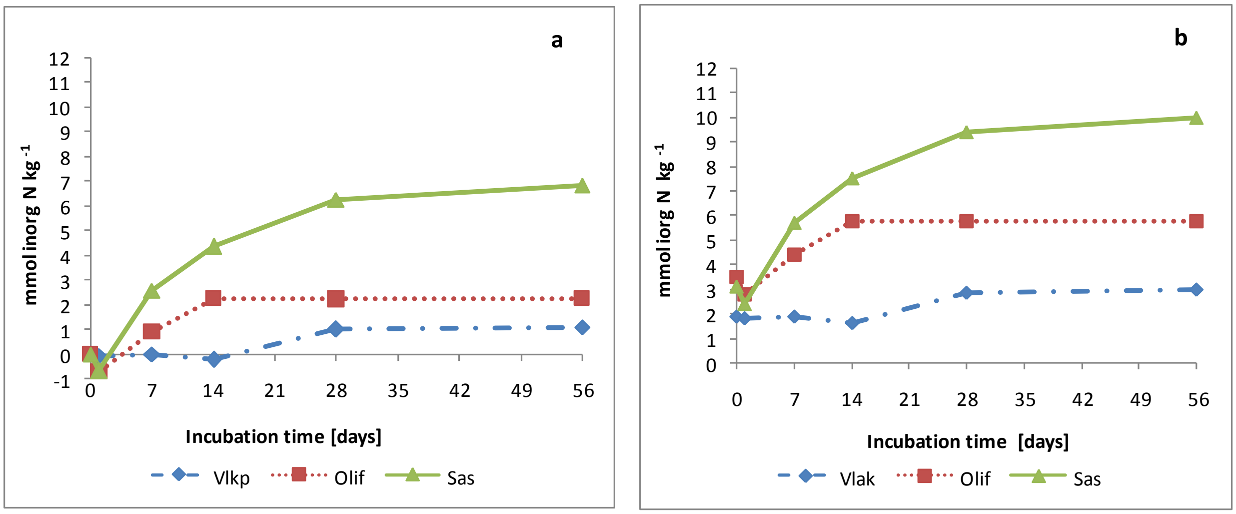

Figure 3.2 Net N mineralized (a) compared to net N released (Mineralization plus N released) from sludges at 25 ^{o}C and -10kPa

Vlakplaas sludge that was anaerobically digested and then paddy dried for an extended time had much higher NH_4^+ and NO_3^- levels (366.7 mmol kg $^{-1}$ and 38.7 mmol kg $^{-1}$) respectively, than sludge perceived to be less stable. The Olifantsfontein sludge, for example, which was unstable activated partially digested and belt pressed containing 66.7 mmol kg $^{-1}$ NH_4^+ and 1.6 mmol kg $^{-1}$ NO_3^- (Table 3.2). The data suggests that at an equivalent loading rate of 10 ton ha^{-1} sludge addition elevated inorganic N levels in the soil between 2 and 5.7 mmol kg^{-1} (Figure 3.2 b). It was expected that the amendment with Vlakplaas sludge will result in the highest initial N levels, however, the data does not reflect this. The reason might have been that KCl extraction have extracted also de dissolved organic N that was much higher for Olifantsfontein having 3714 mmol kg $^{-1}$ of organic N while Vlakplaas had only 735.7 mmol kg $^{-1}$. The availability of N is closely linked to dissolved organic C, which is easily extracted with salt extractant (Silveira, 2005; Dijkstra *et al.*, 2007). This is also the reason why estimated initial inorganic and organic N values differ from the determined values at time T_0.

The net N mineralization was significantly affected by the interaction between temperature and water potential. Sludge N mineralization increased with temperature, and highest net N release was observed at 45 ºC. This was also true for Zaman and Chang (2004), who reported greater N mineralization at 45 ºC and soil moisture around field capacity than at 25 ºC and 5 ºC. These results contradict the optimum temperature range for mineralization (25 ºC – 35 ºC) stated by Doran and Smith (1987), in Figure 2.3. In this study 25 ºC was optimum temperature range for nitrification.
However, these results are in agreement with Quemada and Cabrera (1997), who found the effect of optimal soil water content enhanced with temperature increase. Tajeda *et al.* (2002) also found that N mineralization was higher at 25 ^{o}C than 15 ^{o}C, and that increasing temperature boosted mineralization as well as N losses which can exceed 50 %.

Figures 3.3, 3.4 and 3.5 show N release from Vlakplaas, Olifantsfontein and Sasol sludges, respectively. When comparing them net N release had different trends during the initial stage of incubation. The negative period illustrated in Figure 3.4 and Figure 3.5 was a direct result of the initial low inorganic N content of less stable sludge. For example, the unstable Olifantsfontein sludge contained 2 % inorganic and easily metabolizable N, while stable sludge contained 54 % of available inorganic N. Therefore, in order to mineralize the unstable sludge, microorganisms had to metabolize easily available inorganic N from the soil and that of the sludge as source of energy.

These results are similar to findings of Wang's *et al.*, (2003) that mineralization of organic N in soils amended with bioslids was strongly influenced by its quality and temperature. Probert *et al.* (2005) also observed that at the initial stage of incorporation of organic sources, inorganic N was immobilized even with substrates having C:N ratios less than 20.

3.3.2 Vlakplaas amended soil: Effects of temperature and water potential on the mineralization process

From Figure 3.3 the effect of incubation time, soil temperature and water content on net N release from stable sewage sludge amended soil were evident. The amount of NH_4^+ increased for the first 24 hours of incubation followed by a sharp decrease during the first week for T_1, afterward the decrease was more gradual. This initial increase in the amount of NH_4^+ might have been due to boosting of microbial activity and subsequent ammonification, following moistening of sludge amended samples. At $T_{2,}$ mineralization was insignificant, only nitrification was observed. At high temperature (45 ^{o}C), the amount of NH_4^+ had increased sharply in the first day of incubation, and then an irregular increasing trend was observed. Negligible amounts of NO_3^- were formed indicating that nitrification was inhibited or the nitrate formed was lost. As a result net released N was mainly in ammonium form.

Meanwhile NO_3^- levels increased along the incubation period, as a result of nitrifying bacteria activity. This occurred for all treatment combinations involving T_1 and T_2. However, the trend was different for treatment combinations involving T_3. In which NH_4^+ had increased and no nitrate was formed. The reason is that high temperatures increase mineralization but are unfavorable to nitrifying bacteria.

Results from previous research found that nitrifying bacteria are more sensitive to extreme temperature and moisture conditions than ammonifiers (Sierra *et al.*, 2001; Zaman and Chang, 2004). Another suggestion is that the little NO_3^- which may be formed might have been denitrified and lost by the time samples were aerated and/or the microbe biomass might have assimilated nitrate and nitrite under anaerobic warm conditions (Brady and Weil, 2002).

At 10 °C both inorganic N forms (NH_4^+ and NO_3^-) coexisted up to the end of incubation however, at 25 °C, NH_4^+ was converted to NO_3^- after 28 days of incubation complete conversion of NH_4^+ was observed. The optimum nitrifying conditions is shown by the complete conversion of NH_4^+ to NO_3^-. Ashok *et al*. (2006) found a sharp increase of NH_4^+ for the first fifteen days of incubation at 25 °C, followed by a sharp decline indicating rapid transformation into nitrate-N.

Smith and Tibbett (2004) found an increase in NH_4^+ and decrease in NO_3^- and no net nitrate accumulation, this contradicts with Smith *et al*., (1998a) results which reported net NO_3^- accumulation in soils amended with undigested biolsolids at 25 °C and soil water content at 40 % FC, however, immobilization of inorganic N was significant during the initial stage of incubation.

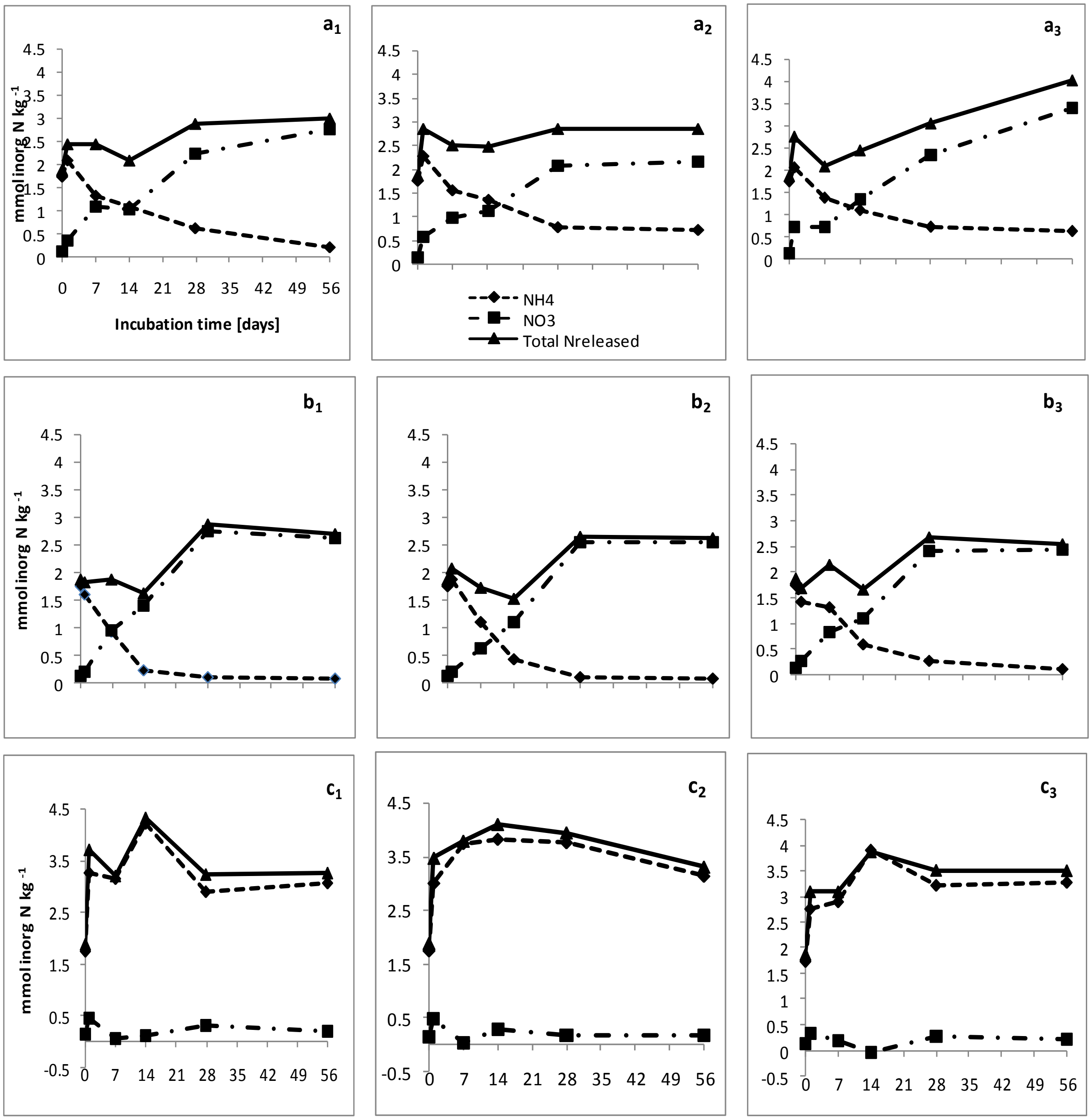

Figure 3.3 Net N released, NH_4^+ and NO_3^- from Vlakplaas sludge during a 56-day laboratory incubation at a temperature and water potential treatments given by letters: **a_1** = T_1W_1, **a_2** = T_1W_2 , **a_3** = T_1W_3; **b_1** = T_2W_1, **b_2** = T_2W_2, **b_3** = T_2W_3; **c_1** = T_3W_1, **c_2** = T_3W_2, and **c_3** = T_3W_3

Based on the ANOVA tables (Appendix A.$_3$), it seems that at 25 ^{o}C the rate of mineralization decreased to the point where it is approaching zero at the end of the 56 day incubation. Significant difference among incubation times were observed at T_2 for nitrification except day 28 and day-56. In general ammonium levels decreased with incubation time and nitrate increased with incubation time except at T_3. The effect of incubation time at T_3 was higher for day 14, lower for day1.

The effect of temperature, water potential and their interactions were statistically significant (Appendix A.$_{2.1}$). Although, every single factor was statistically significant, only interaction effects are discussed as defined in statistics rule. Levels of significance are presented in (Table 3.8), and there were no significant differences in net N released between the high temperature treatments. The T_2W_1 treatment was statistically separated from T_2W_2 and T_2W_3 While the T_1 treatments were all statistically different from each other. Differences for the majority of treatments were highly significant, except T_1W_3 which shows no significant differences with treatments involving T_3. The effect of T_1W_1 treatment on net N release was not statistically different from that of T_2W_1 ($p > 0.05$), however, it was significantly different with T_2W_2 treatment ($p < 0.05$) and it had highly significant difference with the other treatments T_1W_2, T_1W_3, T_2W_3, T_3W_1, T_3W_2, and T_3W_3 ($p < 0.01$).

Table 3.8 Levels of significance between temperature and water potential interaction on N mineralization from Vlakplaas sludge amended soil after a 56- day laboratory incubation.

	T_1W_1	**T_1W_2**	**T_1W_3**	**T_2W_1**	**T_2W_2**	**T_2W_3**	**T_3W_1**	**T_3W_2**
T_1W_2	<.0001**							
T_1W_3	0.0006**	<.0001**						
T_2W_1	0.3809***ns***	<.0001**	0.0041**					
T_2W_2	0.0449*	0.0067**	<.0001**	0.0068**				
T_2W_3	0.0019**	0.1319***ns***	<.0001**	0.0003**	0.1556***ns***			
T_3W_1	0.0031**	<.0001**	0.4476***ns***	0.0219*	<.0001**	<.0001**		
T_3W_2	0.0016**	<.0001**	0.6348***ns***	0.0118*	<.0001**	<.0001**	0.7727***ns***	
T_3W_3	<.0001**	<.0001**	0.3455***ns***	0.0005**	<.0001**	<.0001**	0.0980***ns***	0.1638***ns***

ns *= not significant ($p > 0.05$);* Significant at α= 5% ($p < 0.05$); **Highly significant at α= 1% ($p < 0.01$)*

At low temperature it seems that water potential significantly influenced net N release, but did not show a consistent trend. At the high temperature treatment changes in water potential had no statistical significant effect on the amount of N released, this can be attributed to the difficulty of maintaining the treatment at the specific water potentials.

The effect of high temperature (45 ^{o}C) was superior for NH_4^+ formation and negatively influenced NO_3^- formation (Table 3.9). Net N released increased with temperature (it was 2.77 at $T_1W_1 < 2.90$ at $T_2W_1 < 3.25$ at T_3W_1). The expected trend that net in released will increase as water potentials approach field capacity was only observed for T_2: 2.90 for T_2W_1, 2.46 for T_2W_2, and 2.25 for T_2W_3. The high temperature treatment showed an opposite trend that was the more negative the water potential the higher the observed N released, however the differences were not statistically significant. This could have been an experimental artifact sprouting from the difficulty to maintain the water content constant during the course of the incubation for this specific temperature treatment (45 ^{o}C). Dependency of N release on water potential was significant at 10 ^{o}C treatment.

The effect of treatments with 25 ^{o}C was superior for nitrification. In general, nitrifying bacteria were less active at 45 ^{o}C and more active at 25 ^{o}C whilst ammonifying bacteria, on the other hand, were less active at 25 ^{o}C and more active at 45 ^{o}C. The balance between the proportion of ammonifying and nitrifying bacteria is temperature dependent (Brady and Weil, 2002). Meaning that both ammonifying and nitrifying bacteria can operate concurrently, whenever, the environmental conditions are favorable. However, if the environmental conditions favor one type of bacteria is then when this unbalance on their activity is seen.

Nitrate levels at 10 ^{o}C were comparable with those at 25 ^{o}C, the reason is that at low temperature, if there is a good aeration, nitrifying bacteria might find favorable conditions (oxygen) required for nitrification.

Table 3.9 Ranking and treatment mean comparison of NH_4^+, NO_3^- and net N released, for the Vlakplaas sludge amended soil after 56-days of laboratory incubation.

Treatment	$\mathbf{NH_4^+}$	**Treatment**	$\mathbf{NO_3^-}$	**Treatment**	**Net N released**
			[mmol kg^{-1}]		
$\mathbf{T_3W_3}$	3.28 *(0.04)* ^{A}	$\mathbf{T_1W_3}$	2.99 *(0.07)* ^{A}	$\mathbf{T_3W_3}$	3.50 *(0.17)* ^{A}
$\mathbf{T_3W_2}$	3.13 *(0.34)* ^{AB}	$\mathbf{T_2W_1}$	2.93 *(0.07)* ^{A}	$\mathbf{T_1W_3}$	3.36 *0.12)* ^{AB}
$\mathbf{T_3W_1}$	3.05 *(0.06)* ^{B}	$\mathbf{T_2W_2}$	2.63 *(0.02)* ^{B}	$\mathbf{T_3W_2}$	3.29 *(0.40)* ^{AB}
$\mathbf{T_1W_3}$	0. 80 *(0.02)* ^{C}	$\mathbf{T_2W_3}$	2.60 *(0.04)* ^{BC}	$\mathbf{T_3W_1}$	3.25 *(0.07)* ^{B}
$\mathbf{T_1W_2}$	0.70 *(0.12)* ^{CD}	$\mathbf{T_1W_1}$	2.52 *(0.10)* ^{C}	$\mathbf{T_2W_1}$	2.90 *(0.04)* ^{C}
$\mathbf{T_1W_1}$	0.60 *(0.05)* ^{D}	$\mathbf{T_1W_2}$	1.99 *(0.06)* ^{D}	$\mathbf{T_1W_1}$	2.77 *(0.13)* ^{C}
$\mathbf{T_2W_3}$	0.11 *(0.00)* ^{E}	$\mathbf{T_3W_3}$	0.22 *(0.04)* ^{E}	$\mathbf{T_2W_2}$	2.46 *(0.04)* ^{D}
$\mathbf{T_2W_1}$	0.07 *(0.00)* ^{E}	$\mathbf{T_3W_1}$	0.20 *(0.12)* ^{E}	$\mathbf{T_2W_3}$	2.25 *(0.04)* ^{D}
$\mathbf{T_2W_2}$	0.03 *(0.00)* ^{E}	$\mathbf{T_3W_2}$	0.16 *(0.02)* ^{E}	$\mathbf{T_1W_2}$	2.03 *(0.07)* ^{F}

Treatments means in column followed by the same letter are not statistically different at $\alpha = 5\%$*; LSD for* $NH_4^+ = 0.15$*; LSD for* $NO_3^- = 0.08$ *and LSD for Net N released = 0.21; Figures in brackets denote standard errors.*

3.3.3 Olifantsfontein amended soil: Effects of temperature and water potential on the mineralization process

In general net N mineralization had a similar trend for all water potentials and temperatures; mineralized N was mainly in ammonium form, with little differences in treatments involving T_2. After two weeks of incubation a decrease in NH_4^+ and consequent increase in NO_3^- was observed (Figure 3.4). However, the trend of temperature and water potential effect for Olifantsfontein sludge did differ from the Vlakplaas sludge.

The initial inorganic nitrogen decrease observed after one day of incubation, could be due to microbial flush following sludge application (N negative period). The Vlakplaas sludge treatment did not show this, a possible reason for this could be the high initial inorganic N content. The Olifantsfontein sludge, for example, contained 1.67 % of inorganic N, compared to 55 % for the

Vlakplaas sludge (Table 3.3). It seems that enough easily available N was applied with the Vlakplaas amendment to meet the immediate microbial metabolism demands. However, in the case of Olifantsfontein amended treatment microorganisms were forced to assimilate inorganic N present in the soil and that from the sludge to meet their metabolic demands. Therefore the chance for N immobilization was higher for the less stable sludge, and the same was seen for the Sasol amendment having 4.6% of initial inorganic N.

General trend, during incubation for both NH_4^+ and NO_3^- was the decrease in the initial stage of incubation, followed by a sharp increase in NH_4^+ for the following two weeks then a gradual increase was observed up to the end of incubation at week eight.

A negligible amount of nitrate was formed during the incubation process, except for treatments with T_2 (25 ^oC), where, starting from week two, nitrate formation was observed (Figure 3.4 b_1, b_2 and b_3). These treatments also showed after the second week of incubation, a slightly decrease in NH_4^+.

Treatment T_3W_1 (Figure 3.4 c_1) shows a decrease in NH_4^+ after one day of incubation followed by a sharp increase until week two. Then continued to increase, however, at a slower rate, until week four and decreased for the last four weeks of incubation. With a decrease in NH_4^+ an increase in NO_3^- was expected, however, this did not happen, apparently losses of ammonium might have occurred through volatilization of ammonia (NH_3) or denitrification (N_2O losses).

The absence of nitrification in all treatment with T_1 and T_3 might have been because of the NH_4^+ toxicity to nitrifiers. Although NH_4^+ is required for nitrification, however, when is excessive becomes toxic to *nitrobacter*, and reduces their activity (Brady and Weil, 2002). On average the amount of NH_4^+ was 109 mg kg^{-1} for T_1 and 187 mg kg^{-1} for T_3, however, the amount of NH_4^+ in the treatments was far below the 400 mg kg^{-1}considered the maximum concentration nitrifiers can tolerate (McIntosh and Frederick, 1958). Another reason that may have caused this lack of nitrifications could be that nitrifying bacteria are less competitive than the heterotrophic ammonifying bacteria (Verhagen *et al.*, 1992).

Greater mineralization was observed at 45 ^oC and increased with water potential decrease (7.44; 9.19; 10.5 mmol kg^{-1} for Figure 3.4 c_1 c_2 and c_3) respectively. Nonetheless, greater nitrification was observed at 25 °C and decreased with water potential decrease (1.23; 0.44; 0.78 mmol kg^{-1} for Figure 3.4 b_1 b_2 and b_3) respectively. At 10 °C the net N release increased as soil moisture changed from high negative to low negative water potential ($T_1W_1 > T_1W_2 > T_1W_3$). Sierra *et al.* (2001) found that nitrifying bacteria was more sensitive to changes in water potential than ammonifying bacteria. This supports the results found for stable sewage sludge amended soils where nitrification process was highly temperature dependent.

Theoretically the optimum temperature and water potential combination for mineralization was expected to be T_2W_1, as illustrated in Figure 2.2 and Figure 2.3 (Doran and Smith, 1987). However, in this experiment this combination appeared to be optimum only for nitrification. Zaman and Chang (2004) found that the effect of soil moisture on mineralization was enhanced at lower temperature and the effect of soil moisture on mineralization was masked at higher temperature.

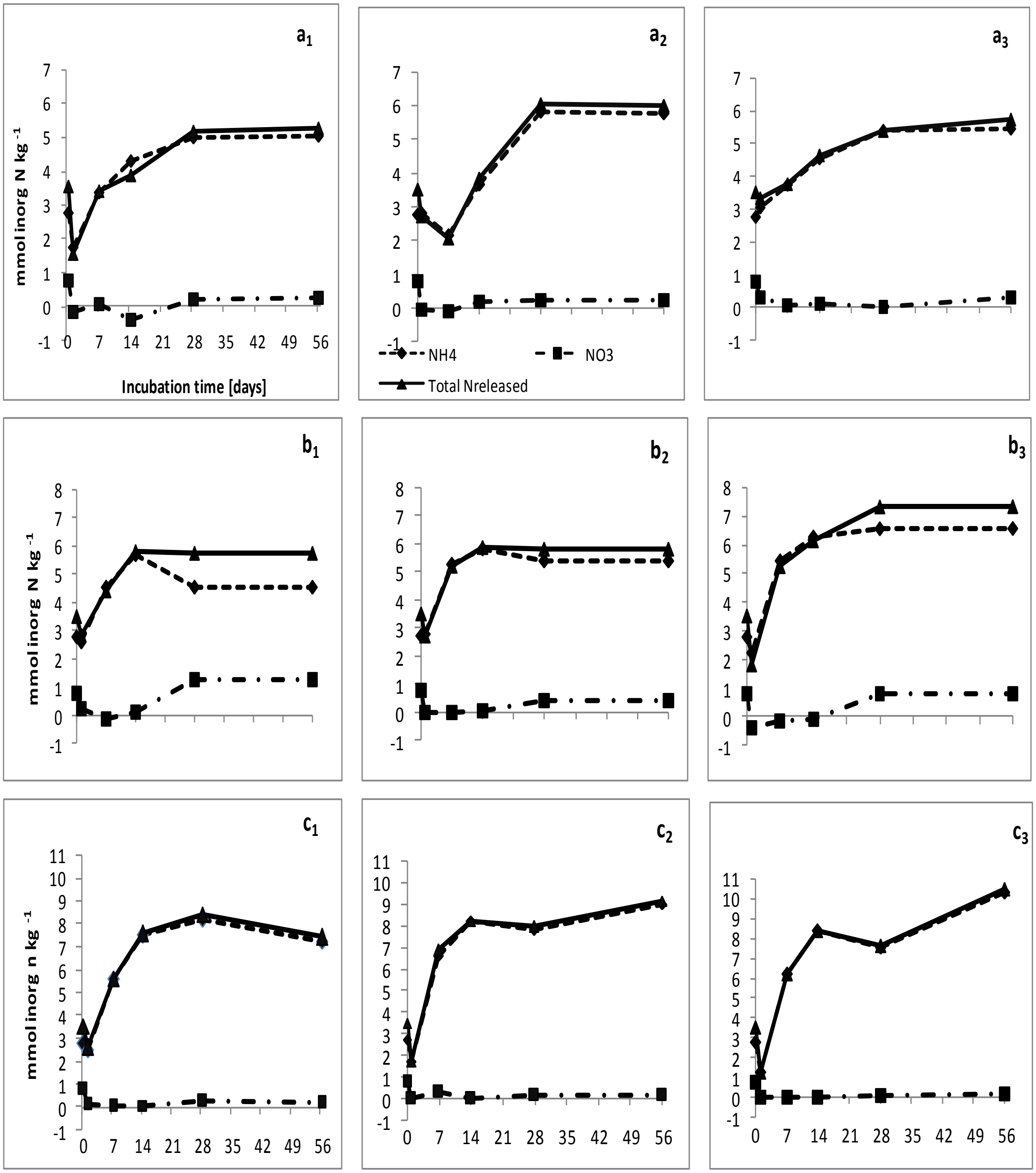

Figure 3.4 Net N released, NH_4^+ and NO_3^- from Olifantsfontein sludge during a 56-day laboratory incubation at a temperature and water potential treatments given by letters: **a_1** = T_1W_1, **a_2** = T_1W_2, **a_3** = T_1W_3; **b_1** = T_2W_1, **b_2** = T_2W_2, **b_3** = T_2W_3; **c_1** = T_3W_1, **c_2** = T_3W_2, and **c_3** = T_3W_3

The ANOVA tables (Appendix A.2.2) shows that temperature, soil water potential and their interactions affected significantly the net N release from Olifantsfontein sludge amended soil. Therefore, only pre-planned interactions were discussed at significance level of 5 % (Table 3.10). In general the higher N release observed for the T_3 treatments where significantly different from that observed from medium and low temperature treatments. The exception was observed for T_2W_3 and T_3W_1 which could not be statistically separated. The treatments involving low temperature and medium temperatures could not be statistically separated.

Table 3.10 Levels of significance between temperature and water potential interaction on N mineralization from Olifantsfontein sludge amended soil after a 56-day laboratory incubation

	T_1W_1	T_1W_2	T_1W_3	T_2W_1	T_2W_2	T_2W_3	T_3W_1	T_3W_2
T_1W_2	0.1706 ***ns***							
T_1W_3	0.0367*	0.4178 ***ns***						
T_2W_1	0.0503 ***ns***	0.5110 ***ns***	0.8756 ***ns***					
T_2W_2	0.0878 ***ns***	0.7100 ***ns***	0.6569 ***ns***	0.7730***ns***				
T_2W_3	0.0004 **	<.0001 **	<.0001**	<.0001**	<.0001**			
T_3W_1	0.0001 **	<.0001 **	<.0001**	<.0001**	<.0001**	0.6434***ns***		
T_3W_2	<.0001 **	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**	
T_3W_3	<.0001 **	<.0001 **	<.0001 **	<.0001**	<.0001**	<.0001**	<.0001**	<.0001**

***ns** = Not significant (p > 0.05);* Significant at α=5% (p < 0.05); ** Highly significant α=1% (p < 0.01)*

The means of all treatments were ordered from the higher to lower value, (Table 3.11). Among treatments lower value of net N release was observed at 10 °C for the interaction T_1W_3 (5.72 mmol kg^{-1}) and the highest value of net N released was found at 45 °C for T_3W_3 (10.5 mmol kg^{-1}), at 25 °C for T_2W_3 net N released was (7.33 mmol kg^{-1}). This scenario agrees with the theory that increased temperature leads to an increase in mineralization of organic material. Tajeda *et al.* (2002) also found that N mineralization was more extensive at 25 °C than 15 °C and that increasing temperature boosted mineralization as well as N losses. However, the lowest values of nitrification were observed at 45 °C and 10 °C, the highest at 25 °C, for example T_1W_1 (0.24 mmol kg^{-1}), T_2W_1 (1.23 mmol kg^{-1}) and T_3W_1 (0.19 mmol kg^{-1}). Under 25 °C nitrification increased with increase in water potential T_2W_1 (1.23 mmol kg^{-1}), T_2W_2 (0.78 mmol kg^{-1}) and T_2W_3 (0.44 mmol kg^{-1}). Sierra *et al.* (2001) found that nitrifiers were more sensitive to changes in water potential than ammonifiers.

Table 3.11 Ranking and mean comparison of NH_4^+, NO_3^- and net N released, for the unstable Olifantsfontein sludge amended soil after 56-days of laboratory incubation

Treatment	**NH_4^+**	**Treatment**	**NO_3^-**	**Treatment**	**Net N released**
			[mmol kg^{-1}]		
T_3W_3	10.4 *(0.45)* ^{A}	**T_2W_1**	1.23 *(0.06)* ^{A}	**T_3W_3**	10.5 *(0.45)* ^{A}
T_3W_2	9.03 *(0.30)* ^{B}	**T_2W_3**	0.78 *(0.06)* ^{B}	**T_3W_2**	9.19 *(0.29)* ^{B}
T_3W_1	7.25 *(0.22)* ^{C}	**T_2W_2**	0.44 *(0.05)* ^{C}	**T_3W_1**	7.44 *(0.18)* ^{C}
T_2W_3	6.55 *(0.08)* ^{D}	**T_1W_3**	0.28 *(0.06)* ^{D}	**T_2W_3**	7.33 *(0.21)* ^{C}
T_1W_1	6.03 *(0.18)* ^{E}	**T_1W_1**	0.24 *(0.05)* ^{DE}	**T_1W_1**	6.27 *(0.24)* ^{D}
T_1W_2	5.78 *(0.02)* ^{E}	**T_3W_1**	0.19 *(0.04)* ^{E}	**T_1W_2**	5.92 *(0.09)* ^{DE}
T_1W_3	5.44 *(0.05)* ^{F}	**T_3W_2**	0.16 *(0.02)* ^{EF}	**T_2W_2**	5.83 *(0.06)* ^{E}
T_2W_2	5.39 *(0.02)* ^{F}	**T_3W_3**	0.12 *(0.02)* ^{F}	**T_2W_1**	5.76 *(0.61)* ^{E}
T_2W_1	4.52 *(0.51)* ^{G}	**T_1W_2**	0.12 *(0.07)* ^{F}	**T_1W_3**	5.72 *(0.04)* ^{E}

Treatment means in column followed by the same letter are not significantly different at $\alpha = 5\%$*; LSD for* $NH_4^+ = 0.32$*; LSD for* $NO_3^- = 0.06$ *and LSD for Net N released = 0.36; Figures in brackets denote standard errors*

Again, as was observed, for Vlakplaas sludge, the Olifantsfontein sludge also showed the highest N mineralization at treatments involving T_3. This is out from the range illustrated by Doran and Smith (Figure 2.3), however, Zaman and Chang, (2004) also found high levels of N mineralized at 40 °C.

3.3.4 The SASOL amended soil: Effects of temperature and water potential on the mineralization process

Figure 3.5 illustrates how inorganic N forms where changing during the 56-day laboratory incubation, under all treatments. Similarly to other unstable sludge from domestic wastewater both ammonium and nitrate decreased after first day of incubation. The negative periods of NO_3^- previously observed for unstable Olifantsfontein sludge amended soil were also observed during initial stage of incubation with the unstable Sasol sludge amended soil. This was also attributed to the initially low inorganic N in unstable Sasol sludge amended soil. Consequently the available NO_3^- was assimilated by microbe population to get energy for their metabolism.

After one day incubation, the trend under interactions with T_1 (Figure 3.5 a_1 – a_3) and with T_3 (Figure 3.5 c_1 – c_3) was similar and differed from interactions with T_2 (Figure 3.5 b_1 – b_3). No nitrification occurred at 10 ^oC and 45 ^oC, however, at 25 ^oC nitrification took place from the second week onwards until the end of incubation.

The absence of nitrification at 10 ^oC and 45 ^oC may be a result of nitrifying bacteria high sensitivity to extreme temperature and to NH_4^+ toxicity. The average amount of NH_4^+ for 10 ^oC and 45 ^oC ranged between 110 – 131 mg kg^{-1}, however, this range was still far from 400 mg kg^{-1}, (McIntosh and Frederick, 1958) and the 800 mg kg^{-1} (Broadbent *et al.*, 1957), found to be the maximum NH_4^+ in soil tolerated by nitrifiers. Other research conducted with the Sasol sludge involving leaching studies also revealed that water soluble arsenic, boron and selenium levels of this sludge is quite high. Especially arsenic is extremely biotoxic and it is reasonable to expect that it will have a negative effect on microbes involved in the mineralization process especially those involved in nitrification.

For T_1 and T_2 interactions with all water potential mineralization decreased from W_1 to W_3. This was in accordance with (Doran and Smith, 1987; Leiros, *et al.*, 1999). As soil water potential decreased (reaching negative levels > - 50 kPa) soil microbe activity was reduced. However, at high temperature the situation was opposite, net N release increased from W_1 to W_3, the same for unstable Olifantsfontein and stable Vlakplaas sludge. Therefore, the effect of water potential on N release was masked at high temperature. Zaman and Chang (2004) support these results, and also found higher mineralization at 40 °C than at 20 °C and at 5 °C.

Considering what is referred in literature, water potential W_1 (- 10 kPa) is close to optimum soil moisture for mineralization. At this water potential, treatments with T_2 (25 °C) resulted in the highest net N release 9.75 mmol kg^{-1}. This was an exception because for other sludge amended soils, higher net N release was at 45 °C. A possible explanation was the existence of 5 % of NH_4^+ in the sludge initially, which was nitrified and incremented the total net N released. Nevertheless, no nitrification was observed at 10 °C and 45 °C, since nitrifiers are depressed at cold and hot conditions (Brady and Weil, 2002). Therefore, greater part of mineralized N remained in NH_4 form at 10 °C and 45 °C treatments.

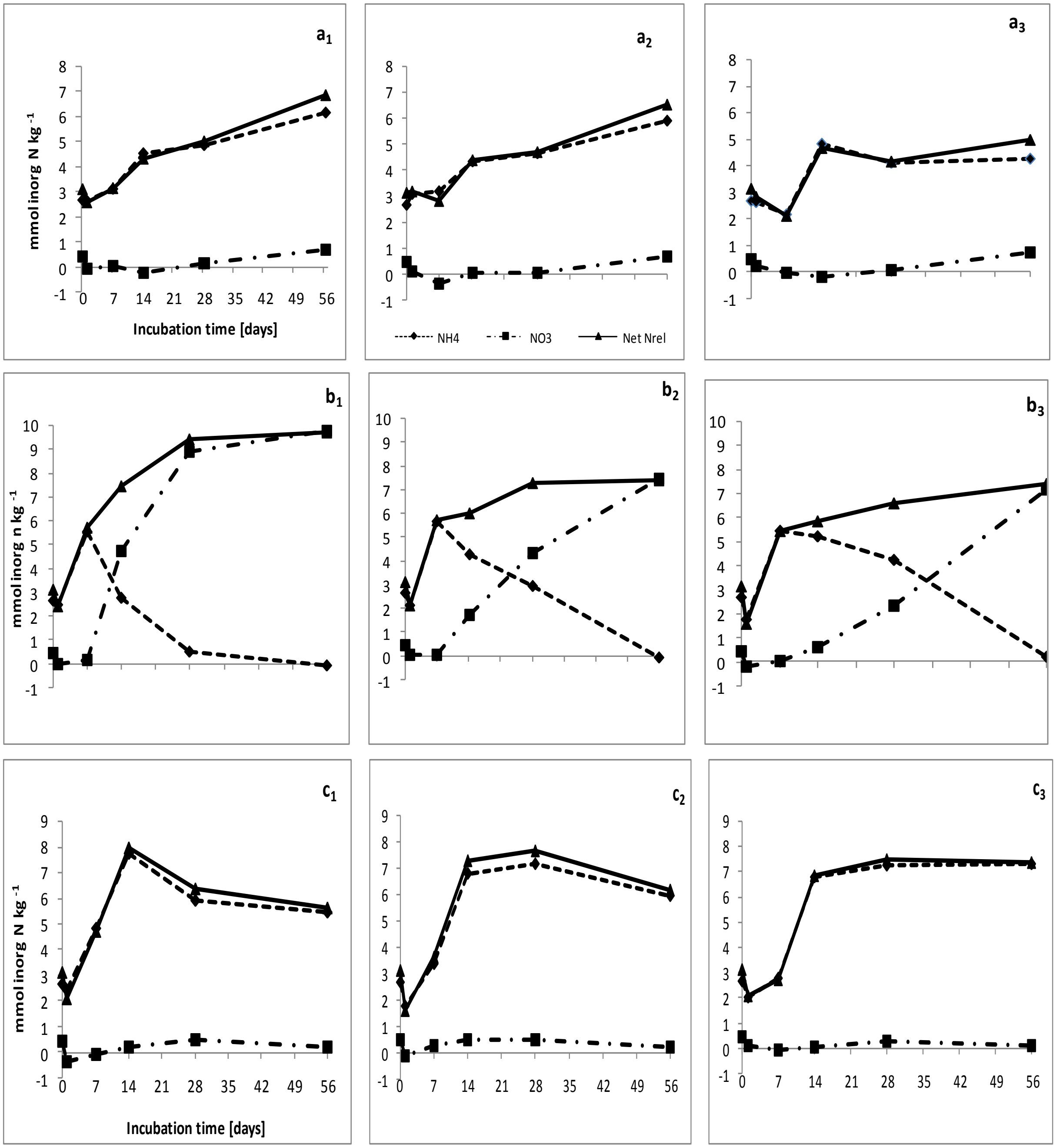

Figure 3.5 Net N released, NH_4^+ and NO_3^- from Sasol sludge during a 56-day laboratory incubation at a temperature and water potential treatments given by letters: **a_1** = T_1W_1; **a_2** = T_1W_2; **a_3** = T_1W_3; **b_1** = T_2W_1; **b_2** = T_2W_2; **b_3** = T_2W_3; **c_1** = T_3W_1; **c_2** = T_3W_2; and **c_3** = T_3W_3

The ANOVA tables (Appendix $A._{2.3}$) shows that temperature, soil water potential and their interactions affected significantly the net N released from unstable Sasol sludge amended soil. Only interaction effect is discussed by rule. Table 3.12 illustrates treatments differences, their level of significance and which were not statistically different. For example treatments T_1W_3, T_2 W_1 and T_3W_1 had highly significant differences with all other treatments, even among them were statistically different.

Table 3.12 Levels of significance between temperature and water potential interaction on N mineralization from Sasol sludge amended soil after a 56- day laboratory incubation

	T_1W_1	**T_1W_2**	**T_1W_3**	**T_2W_1**	**T_2W_2**	**T_2W_3**	**T_3W_1**	**T_3W_2**
T_1W_1								
T_1W_2	0.0729*ns*							
T_1W_3	<.0001**	<.0001**						
T_2W_1	<.0001**	<.0001**	<.0001**					
T_2W_2	0.1449*ns*	0.0030**	<.0001**	<.0001**				
T_2W_3	0.1449*ns*	0.0030**	<.0001**	<.0001**	1.0000*ns*			
T_3W_1	<.0001**	<.0001**	0.0020**	<.0001**	<.0001**	<.0001**		
T_3W_2	0.0008**	0.0505*ns*	<.0001**	<.0001**	<.0001**	<.0001**	0.0105*	
T_3W_3	0.0157*	0.0002**	<.0001**	<.0001**	0.2680*ns*	0.2680*ns*	<.0001**	<.0001**

ns *= Not significant* $(p > 0.05)$;* *Significant at α= 0.05* $(p < 0.05)$; ** *Highly significant α= 0.01*$(p < 0.01)$

Treatment means for NH_4^+, NO_3^- and net N released were listed orderly from high to low compared using the least significant difference (LSD) Table 3.13. Treatments were compared along the column; those with same letter had similar effect on mineralization or nitrification, and with different letters affected differently. Interaction on suboptimal conditions of temperature and water potential (T_1W_3), as expected, gave lower net N released (5.0 mmol kg^{-1}) and the interaction T_2W_1 considered as the optimal condition for nitrification performed well giving the highest amount of both NO_3^- and the net N release (9.75 mmol kg^{-1}), was found to be the best combination for net N release. This was evident that ammonified N was readily nitrified. Therefore net N released after 56-

days of incubation was in nitrate form. However, the T_3W_3 treatment was superior with highest NH_4^+ mineralized (7.28 mmol kg^{-1}) and insignificant NO_3^- similarly to stable Vlakplaas and unstable Olifantsfontein sewage sludge amended soils.

Table 3.13 Ranking and mean comparison of NH_4^+, NO_3^- and net N released, for Sasol sludge amended soil after 56-days of laboratory incubation

Treatment	**NH_4^+**	**Treatment**	**NO_3^-**	**Treatment**	**Net N released**
	[mmol kg^{-1}]				
T_3W_3	7.28 *(0.09)* ^{A}	**T_2W_1**	9.75 *(0.55)* ^{A}	**T_2W_1**	9.75 *(0.38)* ^{A}
T_1W_1	6.15 *(0.04)* ^{B}	**T_2W_2**	7.47 *(0.25)* ^{B}	**T_3W_3**	7.47 *(0.10)* ^{B}
T_3W_2	5.97 *(0.08)* ^{C}	**T_2W_3**	7.18 *(0.24)* ^{B}	**T_2W_2**	7.10 *(0.26)* ^{C}
T_1W_2	5.87 *(0.13)* ^{C}	**T_1W_3**	0.69 *(0.03)* ^{C}	**T_2W_3**	7.10 *(0.26)* ^{C}
T_3W_1	5.44 *(0.12)* ^{D}	**T_1W_1**	0.69 *(0.10)* ^{C}	**T_1W_1**	6.83 *(0.21)* ^{C}
T_1W_3	4.28 *(0.10)* ^{E}	**T_1W_2**	0.65 *(0.02)* ^{C}	**T_1W_2**	6.50 *(0.10)* ^{D}
T_2W_1	0.21 *(0.03)* ^{F}	**T_3W_1**	0.20 *(0.02)* ^{D}	**T_3W_2**	6.13 *(0.12)* ^{E}
T_2W_2	0.00 *(0.00)* ^{G}	**T_3W_2**	0.19 *(0.02)* ^{D}	**T_3W_1**	5.63 *(0.12)* ^{F}
T_2W_3	0.00 *(0.00)* ^{G}	**T_3W_3**	0.10 *(0.02)* ^{D}	**T_1W_3**	5.00 *(0.20)* ^{G}

Treatment means in column followed by the same letter are not statistically different at $\alpha = 5\%$; LSD for $NH_4^+ = 0.09$; LSD for $NO_3^- = 0.28$ and LSD for Net N released = 0.26; Figures in brackets denote standard errors

3.3.5 Partial N mass balance

The term partial used means that inorganic N losses were not included (volatilization and immobilization). Based on the sludge characterization results (Table 3.1), conversion was made into an easily comparable unit for nitrogen specimens [mmol per kg] for all N-forms (NH_4^+, NO_3^-, Org N, Tot N) (Table 3.2). Organic N values were obtained by subtracting total inorganic N released from total N. The assumed total net inorganic N (report only ammonium and nitrate plus nitrite) because volatilization was not measured in this study. However, with this approach organic N was overestimated as a result of an underestimation of N mineralized (not accounting for N losses through immobilization and /or volatilization). Organic N mineralized was obtained by subtracting Org N at instant time (t_i) from Org N at time (t_0).

Table 3.14 illustrating the N mass balance shows that net N release from Vlakplaas sludge amended soil varies in the range of 1.87 – 3.50 mmol kg^{-1} with temperature increase. On average 26.2 % of the total organic N was mineralized after 56 days of laboratory incubation, however, considering the total inorganic N released the trend for potentially available N supply was 47.8 % of total N at 10 ºC, 50.0 % at 25 ºC and 56.0 % at 45 ºC. Meaning that, Vlakplaas sludge may supply 38.8 mg kg^{-1} of N at 10 ºC, 40.6 mg kg^{-1} of N at 25 ºC and 45.6 mg kg^{-1} of N at 45 ºC.

At 10 ºC a decrease in the amount of NH_4^+ was registered and an increase in NO_3^- was observed, as a result of both mineralization and nitrification. Although at 25 ºC a decrease in NH_4^+ and an increase in NO_3^- were also registered as a result of nitrification. High level of nitrification at 25 ºC was reflected by the higher disappearance rate of NH_4^+. At 45 ºC mineralization was high and almost all N mineralized was in form of ammonium, and the potentially available N was high (56.0 %) compared to that at 10 and 25 ºC. No nitrification was observed suggesting NO_3^- assimilation or N_2O losses.

Table 3.14 Partial N mass balance for the 56- day laboratory incubation with Vlakplaas sludge

Treatments	[mmol kg^{-1}]							%	
	Tot N T_0	**NH_4^+**	**NO_3^-**	**net N released**	**net N mineralized**	**Org N**	**Org N mineralized**	**Mineralizable org N**	**Potentially available N**
T_0	5.8	1.74	0.13	1.87	-	3.93	-	-	32.2
T_1W_1	5.8	0.60	2.17	2.77	0.90	3.03	0.90	22.9	47.8
T_1W_2	5.8	0.70	1.99	2.69	0.82	3.11	0.82	20.9	46.4
T_1W_3	5.8	0.79	2.57	3.36	1.49	2.44	1.49	37.9	57.9
T_2W_1	5.8	0.07	2.83	2.90	1.03	2.90	1.03	26.2	50.0
T_2W_2	5.8	0.07	2.55	2.62	0.75	3.18	0.75	19.1	45.2
T_2W_3	5.8	0.06	2.44	2.50	0.63	3.30	0.63	16.0	43.1
T_3W_1	5.8	3.06	0.20	3.26	1.39	2.54	1.39	35.4	56.2
T_3W_2	5.8	3.13	0.16	3.29	1.42	2.51	1.42	36.1	56.7
T_3W_3	5.8	3.28	0.22	3.50	1.63	2.30	1.63	41.5	60.3

From Table 3.15 the partial N mass balance shows that net mineralized N from unstable Olifantsfontein sludge amended soil varied in the range of 3.51 – 10.5 mmol kg^{-1} with temperature increase. At higher temperature (45 ºC) and water potential of -10 kPa the total net mineralized N increased in the order of 46.5 % of total N. At 25 ºC after a 56- day incubation the potentially available N was 35.9 % of the total N, which is equivalent to 17.9 % of mineralizable organic N. Meaning that, Olifantsfontein sludge may supply in average 80.4 mg kg^{-1} of N at 25 ºC and 104.1 mg kg^{-1} of N at 45 ºC.

At 25 ºC, mineralization seemed to be less compared to all other treatment suggesting unfavourable condition at first glance. However, this was not the case the low levels of NH_4^+ indicate that conditions were optimal for the oxidation of it to higher oxidation status of N (N +3 and N+5). What happened is that the NH_4^+ formed was therefore very effectively nitrified as indicated by the relatively high amount of NO_3^- compared to other treatments.

Table 3.15 Partial N mass balance for the 56- day laboratory incubation with Olifantsfontein sludge

Treatments	[mmol kg^{-1}]							%	
	Tot N T_0	**NH_4^+**	**NO_3^-**	**Net N released**	**Net N mineralized**	**Org N**	**Org N mineralized**	**Mineralizable org N**	**Potentially available N**
(T_0)	16.0	2.75	0.76	3.51	-	12.5		-	21.9
T_1W_1	16.0	6.02	0.24	6.26	2.75	9.74	2.75	22.0	39.1
T_1W_2	16.0	5.44	0.25	5.69	2.18	10.3	2.18	17.5	35.6
T_1W_3	16.0	5.79	0.10	5.89	2.38	10.1	2.38	19.1	36.8
T_2W_1	16.0	4.52	1.23	5.75	2.24	10.3	2.24	17.9	35.9
T_2W_2	16.0	5.39	0.44	5.83	2.32	10.2	2.32	18.6	36.4
T_2W_3	16.0	6.55	0.78	7.33	3.82	8.67	3.82	30.6	45.8
T_3W_1	16.0	7.25	0.19	7.44	3.93	8.56	3.93	31.5	46.5
T_3W_2	16.0	9.03	0.16	9.19	5.68	6.81	5.68	45.5	57.4
T_3W_3	16.0	10.4	0.12	10.5	7.01	5.48	7.01	56.1	65.8

Table 3.16 shows that net mineralized N from Sasol sludge amended soil ranged from 3.11 – 9.75 mmol kg^{-1} showing a maximum at 25 ºC. This maximum net N released was not a result of organic N mineralization only, was also from nitrification of the initially existing NH_4^+ and the NH_4^+ coming from mineralization. Whereas, for 10 ºC and 45 ºC treatments net N mineralized was a result of N mineralization, as nitrification was insignificant.

The potentially available N at optimal mineralization condition was 41.1 % of the total N which is equivalent to 32.2 % of the total sludge organic N. The trend observed with potentially available N in average was 28.8 % at 10 ºC, 41.1 % at 25 ºC and 27.0 % at 45 ºC. Meaning that, the Sasol sludge amended soil sample may supply 85.4 mg kg^{-1} of N at 10 ºC, 136 mg kg^{-1} of N at 25 ºC and 89.6 mg kg^{-1} of N at 45 ºC.

N mineralization seemed to be minimum at 25 ºC and higher at 45 ºC, however, the total N released, as well as the potential available N were higher at 25 ºC. Because at 25 ºC nitrifying bacteria encountered optimal conditions for their activity, therefore the NH_4^+ was converted to NO_3^- quickly.

Table 3.16 Partial N mass balance for the 56- day laboratory incubation with Sasol sludge

Treatments	[mmol kg^{-1}]							%	
	Tot N	**NH_4^+**	**NO_3^-**	**Net N released**	**Net N mineralized**	**Org N**	**Org N mineralized**	**Mineralizable org N**	**Potentially available N**
(initial N)	23.7	2.67	0.44	3.11	-	20.6	-	-	13.1
T_1W_1	23.7	6.15	0.69	6.84	3.73	16.9	3.73	18.1	28.8
T_1W_2	23.7	5.87	0.65	6.52	3.41	17.2	3.41	16.6	27.5
T_1W_3	23.7	4.27	0.69	4.96	1.85	18.7	1.85	8.98	20.9
T_2W_1	23.7	0.00	9.75	9.75	6.64	14.0	6.64	32.2	41.1
T_2W_2	23.7	0.00	7.47	7.47	4.36	16.2	4.36	21.1	31.5
T_2W_3	23.7	0.20	7.18	7.38	4.27	16.3	4.27	20.7	31.1
T_3W_1	23.7	5.44	0.20	5.64	2.53	18.1	2.53	12.3	23.8
T_3W_2	23.7	5.97	0.19	6.16	3.05	17.5	3.05	14.8	26.0
T_3W_3	23.7	7.28	0.09	7.37	4.26	16.3	4.26	20.7	31.1

3.3.6 Mineralization rate constant and half life time

A common approach in N modeling is to generate N release and mineralization parameters at optimum conditions for mineralization process (Serna and Pomares, 1992; Zaman *et al* 1999; Hernandez *et al.*, 2002). The same approach was followed here, data collected at 25 ^{o}C and water potential of approximately -10 kPa was used to assess the kinetics of N release from sludge amended soils. In order to determine the rate order of N release (pseudo first order or zero order) data from mineralization was presented in terms of natural logarithm of organic Nitrogen decay as a function of time then plotted against time to assess the linearity of this relationship, which is the diagnostic test for first order reaction (Figure 3.6). The organic N was taken as the difference between initially existing org N and the net N released at each incubation period. Thereafter the organic N values were converted into natural logarithm to provide data to compute the rate constants based on *equation 13*

The linear regression coefficient revealed that N mineralization for Olifantsfontein and Sasol were reasonable well approximated during the first 28 day utilizing first order kinetics (Figure 3.6 a). The organic N decay in the Vlakplaas amended soil seemed to follow first order kinetics during the whole incubation period (Figure 3.6 b). These results support findings of various other authors (Serna and Pomares, 1992; Benbi and Richter, 2002; Hernandez *et al.*, 2002; Hseu and Huang, 2005).

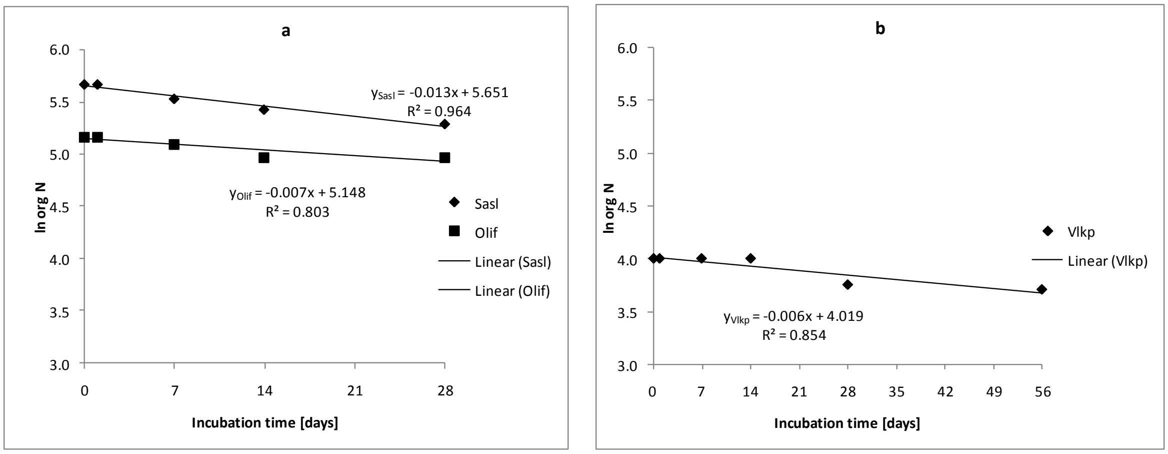

Figure 3.6 The natural logarithm of organic N decay and estimated rate constants (slope of graphs) for Sasol and Olifantsfontein sludge (a) compared to that of Vlakplaas (b)

The Vlakplaas sludge amended soil had the lowest mineralization rate constant (0.042 week $^{-1}$) and longest corresponding half life time (116 days) while Sasol sludge amended soil exhibited the highest rate constant (0.093 week $^{-1}$) and shortest half life time (58 days) (Table 3.17). There was small diference between the Vlakplaas and Olifantsfontein sludge amended soil having a rate of (0.049 week $^{-1}$) and half life time of (98 days). The reason could be that both are municipal sludges therefore presenting simillar composition on organic compounds. These values are similar to Stantford and Smith, (1972) findings, ranging from (0.035 – 0.095 week^{-1}) in 39 samples studied. Also Hseu and Huang (2005) reported that anaerobically digested sludges had rates from 0.047 – 0.075, and rates of 0.047 – 0.105 for aerobically digested sludges. Differently high mineralization rates were reported 0.089 – 0.883 week^{-1} (Serna and Pomares, 1992) and 0.228 – 1.140 week (Hernandez *et al*., 2002). Van Niekerk *et al*., (2005) findings from the Olifantsfontein sludge where relatively high (0.212 day^{-1}). These results also support findings by Dou *et al.,* (1996), who reported the goodness of fit of different kinetic models dependent on incubation period, where single first-order model provided good fit of data for incubation period (≤ 15 weeks); and double first-order model provided better results for incubation period (> 15 weeks.

Table 3.17 N mineralization rate constants and half life times of the fast cycling "pool"

Sludge type	C/N	Total Org N [mg kg $^{-1}$]	k (Day $^{-1}$)	k [Week $^{-1}$]	Half life time [days]
Vlakplaas	6.0	55.0	-0.006	-0.042	116
Olifantsfontein	4.5	175	-0.007	-0.049	98
Sasol	5.0	288	-0.013	-0.093	58

The incubation time was selected based on previous studies done on the sludge collected from Olifantsfontein WCW. This study, showed that after 42 days the rate of N mineralization approached zero and ammonium formation was virtually negligible after 28 days (Van Niekerk *at al*., 2005). Also from Figure 3.4 it's clearly reflected that after 28 days mineralization has reached slow release pool. However, to get some quantification on the potential sizes of slower cycling N pool the approach of Smith *et al*., (1998c) was used. The N pool sizes were estimated at 26 % of total organic N applied for rapid release pool (N_1) and 42 % of total organic N applied for slow release pool (N_2) based on the findings (Table 3.18)

Table 3.18 Estimated sizes of N pools of different types of sludge investigated

Sludge source	Tot Org N	(mg kg $^{-1}$)			
	(mmol kg $^{-1}$)	Tot Org N	Rapid release pool	Slow release pool	Resistant pool
Vlakplaas	3.93	55.0	14.3	23.1	17.6
Olifantsfontein	12.5	175	45.5	73.5	56.0
Sasol	20.6	288	74.9	121	92.3

It is reasonable to expect that a double first order kinetic model by Molina *et al.*, (1980) would better estimate the rate constant to account for the existence of different organic N pools, the rapidly (N_1) and slowly (N_2) N pools (Dou *et al.*, 1996; Hseu and Huang, 2005; Smith *et al.*, 1998c; Benbi and Richter, 2002). However, it was not possible from this study to obtain rate constants of slower cycling pools.

Checking the Van't Hoff equation: testing the temperature coefficient (Q_{10}) and mineralization rate considering 25°C as optimal incubation temperature

$$N = e^{k(t-T)};\quad Q_{10} = e^{10k}$$

Table 3.19 Mineralization rate and temperature coefficient

Sludge source	k	$Q_{15}=e^{15k}$	$N=e^{k(10-25)}$	$Q_{10}=e^{10k}$	$Q_{20}=e^{20k}$	$N=e^{k(45-25)}$
Vlakplaas	-0.006	0.91	1.09	0.94	0.89	0.89
Olifantsfontein	-0.007	0.90	1.11	0.93	0.87	0.87
Sasol	-0.013	0.82	1.22	0.88	0.77	0.77

N mineralization rate decreases with increase in temperature and increase with decrease in temperature relative to the optimal temperature, and the temperature coefficient increases with negative shifting of temperature. These results are not in agreement with Vant'Hoff's assumptions that mineralization rate is twofold when temperature shifts 10 °C in a temperature range between 5 to 35 °C.

3.4 General discussion

Sewage sludge land application has been recognized as a viable alternative for disposal, because with this strategy two problems can be solved at once: Soil productivity (soils are restored, soil fertility enhanced) and environment (potential environmental pollution minimized).

The South Africa's guideline establishes an upper application limit of 10 ton per hectare per year regardless of the type of sludge produced under different process of treatment. What are the implications of the single application dose?

From the obtained net N release results, sludge stability (physical aspect depending on the treatment process) and initial N content shows significant differences in N loads. This difference will continuously be there as a result of different sources of sewage effluent and different treatment processes.

For instance, with the upper limit of 10 t ha^{-1} year^{-1} results from the three tested sludges, total N loaded based on total N content was 193 kg for Vlakplaas sewage sludge, 533 kg and 791 kg for unstable Olifantsfontein and Sasol sludges respectively. From which the potential available N was 96.5, 192, and 324 kg for the same order. Therefore, it's important for decision makers to consider these aspects (stability and total N content) for establishing an effective sludge land application rate. Future refinements of sludge utilization guidelines in South Africa should include upper limits for nitrogen application based on potential available N rather than general sludge loading rates.

The potential for environment nitrate pollution is high for unstable sludges because of their high levels of N content. Therefore, regulations on disposal management strategy need to be reviewed and re-established. Although disposal of sludge as land application seems to be safer; monitoring is important to prevent accumulation of organic pollutants on receiving plots. Site specific modelling N dynamics is an important tool for strategic sludge land application management.

Although, sewage sludge could help in improving soil productivity, its utilization is still limited to urban and peri-urban areas, where functional sewage system and facilities for waste water treatment are located. Therefore, many African countries are still far from exploiting the advantages brought up by sewage sludge land application. For example, in Mozambique, like many other countries, waste-water streams are still dumping to the sea. Remote rural areas have no sanitary facilities. Therefore even if a project for establishing a wastewater treatment company could appear in the big cities to divert the waste streams from dumping into the sea, produced sewage sludge will not help much for subsistence farmers, practicing their agriculture in rural areas because transport of high loads of sewage sludge can be costly and not economically viable.

In remote areas a problem of low fertility with consequent decrease in crop yields faced by rural farmers persists, therefore, other alternatives to counteract soil fertility decline must be found, such as the use of green manure as cover crop or mulching and animal manure. Results from this study are of concern to South Africa's environment, however, for Mozambique, lacking the high investments for establishment of municipal wastewater treatment plants; this work serves as a tool to investigate the dynamic of nutrients from other organic sources in order to recover and enhance soil productivity.

3.5 Conclusions and recommendations

3.5.1 Conclusions

Results from this study, emphasized the importance of sewage sludge as viable soil amendment as a source of nitrogen, given the fact that, it contains high organic matter with ability to release inorganic N nutrient (ammonium and nitrate essential for plant growth).

Temperature and soil water interaction as well as sludge stability, had a significant influence on N release, thus on N availability from sludge amended soils. Greater mineralization was observed at 45 ^{o}C and increased with water potential decrease; nonetheless, greater nitrification was observed at 25 ^{o}C and decreased with water potential decrease. During the incubation period nitrification was suppressed in the Olifantsfontein amended soil. And the N released at 45 ^{o}C was mainly in the NH_4^+ form. Although, at 25 ^{o}C and -10kPa the potential for N release was high for Vlakplaas, the amount of N released was less relative do Olifantsfontein, as a result of initial sludge N content.

Based on the potential N availability Vlakplaas would supply about 96.5 kg inorganic N that represents 27.5% of organic N, Olifantsfontein 192 kg and Sasol 323 kg that represent respectively 34.8, and 38 % of organic N mineralized. Results from this study are in agreement with van Niekerk's (2005) findings, who found that organic N mineralizable from Olifantsfontein was 33.6%. Also according the guidelines for the permissible utilization and disposal of sludge in South Africa the about 30% of organic N is mineralized during the first year. Hernandez *et al.*, 2002 also found that sewage sludge organic N mineralized ranges from 30 to 41%.

There was a clear difference between the mineralisation rate of industrial and municipal sludge. The Vlakplaas sludge amended soil had the lowest mineralization rate constant (0.042 week $^{-1}$) and the longest corresponding half life time (116 days) while Sasol sludge amended soil exhibited the highest rate constant (0.093 week $^{-1}$) and shortest half life time (58 days). Based on the half life times it is concluded that the persistence of stable sludges is longer than unstable Olifantsfontein making it potentially a more efficient source in releasing plant nutrients gradually. Unstable Sasol sludge easily release its N increasing the potential for contamination of the ash dumping sites.

3.5.2 Recommendations

Mineralization and nitrification rates, rate constants of N mineralization and half-life time of sludge decay are useful parameters for modeling. Therefore to improve the N use efficiency from applied sewage sludge and minimize adverse impacts on the environment, it is recommended to establish specific application rates based on the kinetics of N release form sludge.

Determination of inorganic N released based on KCl extractable ammonium (NH_4^+) and nitrate (NO_3^-) may not reflect the release of inorganic only, because KCl does not necessarily discretely extract inorganic N. It is quite possible that organic N present in the sludge, that is soluble in a polar solvent, will also be extracted along with the inorganic N. Therefore determination of organic N after each incubation time is recommended to refine mineralisation estimates and also to predict the dynamics of organic N.

Based on the potential N availability, rate constant and half life Vlakplaas sludge is more effective as it releases N for land application supplying at all temperature range tested amounts of net N release not exceeding the N requirement of the most common crops and at slower release rate. The Olifantsfontein sludge has also a lower release rate; however, may easily exposes the environment to potential nitrate pollution as a result of high levels of total N. It is recommended that sewage sludge be incorporated in soils at least one month before planting to assure for available inorganic N at the stage of crop development.

3.6 Limitations

Losses of N were not captured so the N mass balance given in this study was partial; organic N used for rate constant was an approximation of shorter term N release and longer term predictions only gives an indication of longer term trends.

The half lifetimes may not reflect the reality because the pool sizes were estimated, it is suggested that in the next experiments be determined.

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CHAPTER IV: SAMPLE HANDLING STRATEGY

Handling of sewage sludge amended soil samples for nitrate and ammonium analysis

ABSTRACT

Field validation of mineralization and nitrification rates is essential for accurate prediction and modeling of environmental fate of nitrogen entering the soil system through sewage sludge application. Mineralization and nitrification are ongoing processes, therefore sampling and handling will determine how representative and accurate the determined nitrate and ammonium speciation is to that in the soil at the point in time of sampling. To establish an acceptable sample handling procedure for nitrate and ammonium determinations in sewage sludge amended soils three sample handling strategies (Direct Field Extraction-DFE, Field Drying and Extraction-FDE and Laboratory Drying and Extraction-LDE) were tested. The amounts of nitrate and ammonium speciation determined through DFE- procedure was expected to be the best procedure for equivalent amounts present in soil at the point in time of sampling. Samples were collected from sewage sludge amended fields that received recently (2 seasons) and long period (> 4 seasons) sewage sludge applications. Samples were replicated four times. A known soil volume that passed through a 2 mm sieve was directly transferred to containers with 100 cm^3 of 2*M* KCl solution just after sampling (DFE). Other sub samples were left to air-dry and immediately transferred to containers containing extract solution in the field (FDE). These soil suspensions were transported immediately to the laboratory, extracted and analysed for nitrate and ammonium using the Kjeldahl method. Other sub samples were taken to the Soil Science Laboratory of Pretoria University, air dried for 24 hours sieved through a 2 mm sieve, extracted with a100 cm^3 2 *M* KCl solution (LDE) and analysed for nitrate and ammonium. Sample handling procedure significantly influenced the content of NO_3^- and NH_4^+ speciation. The average NH_4^+ content for DFE was 56.4 mg kg^{-1} (dry land) and 59.5 mg kg^{-1} for LDE. The average NO_3^- content for DFE was 394 mg kg^{-1} (dry land) and 569 mg kg^{-1} for LDE. Analytical results showed that laboratory drying resulted in an overestimation of soil nitrate content and no significant difference for NH_4^+ content. Artifacts introduced by long drying period resulted in an increase of mineralization as well as nitrification in sewage sludge amended samples. Therefore DFE procedure revealed to be the suitable sampling strategy for sewage sludge amended soils.

Keywords: *Soil sampling strategy, sewage sludge, nitrate, mineralization, and nitrification.*

4.1 Introduction and background

The truthfulness of a laboratory soil test results is not only influenced by the methods of analysis and technician's ability but is also influenced by the quality of the soil sample, by means of representative sample collection (Franzen and Cihacek, 2004; Ferro S., 2004). Inadequate sampling and sample handling procedure may result on misleading interpretation of laboratory results. Therefore sampling should be considered seriously as well as soil sample handling to provide representative, consistent and reliable laboratory results.

The dynamic of soil processes cause continuous changes in the forms, quantities and availability of plant nutrients over time. On the other hand the inorganic N forms in particular nitrate (NO_3^-) which is easily mobile in soil solution, cause modifications in soil nitrate and ammonium concentrations throughout the time. This mobility brings in difficulties for modeling N fate in soils especially on soils amended with sewage sludge or other organic source.

To reduce this drawback it is essential to know and understand phenomenon's that might cause soil nutrients value to change between sampling and laboratory testing, as well as, planting time to avoid misinterpretations of laboratory results (Self and Soltanpour, 1997).

Besides environmental conditions, transformations of nitrogen forms in the soil-plant-environment systems are time dependent. The soil micro-organisms composition exerts a big influence on the balance of NH_4^+ and NO_3^- quantities in soil (Verhagen *et al.*, 1992). Nitrifiers are found to be less competitive than heterotrophic bacteria for ammonium. Therefore, the amount of NH_4^+ and NO_3^- determined depends on how samples were handled after collection up until the stage of laboratory analysis.

The ability to extrapolate soil laboratory results to the field conditions depends not only on the fertility expert experience but on how representative the soil sample is to that of real field condition (Ferro S., 2004; Franzen and Cihacek, 2004). For that reason sampling and sample handling for a sewage sludge amended soil, where organic compounds are in continuous mineralization, needs special attention to keep the representativeness of nutrient contents.

Selection of sample handling procedure must be based on the type of species to analyse and the purpose of the results (Self and Soltanpour, 1997). According to Tack and Verloo (2001) sample handling guideline is strongly dependent on the ultimate goal of a particular sampling.

According Self and Soltanpour (1997) for nitrate analyses soil samples should be air dried within 12 hours after sampling, to prevent microbial activity from mineralizing organic materials and causing changes on the ultimate soil ammonium and nitrate contents.

Lack of standard handling procedure for sewage sludge amended soils may lead to wrong interpretations in terms of N status. A standardized procedure for sample handling is necessary to ensure representativeness and reliability of laboratory test results (Theocharopoulos *et al.*, 2001). Standardization of sample handling procedure becomes an urgent need to facilitate interpretation and comparability of data among different laboratories. Therefore adequate sample handling of sewage sludge amended soils are crucial for effective recommendations on the management of sewage sludge land application.

Since the N transformations among organic and inorganic forms are governed by biological soil environment, there is a need to suppress the microbial activity from the sampling stage to the instant of laboratory analysis in order to keep the real amount of NH_4^+ and NO_3^- present in soil at the point in time of sampling (Wollum, 1994).

According the equation $NH_4^+ + OH^- = H_2O + NH_3$, ammonium in soil solution is highly dependent on the pH of the soil. Ammonium may volatilize liberating ammonia as soil dries out driving the reaction equilibrium to the right stimulating NH_4^+ decrease. Ammonium and nitrate content in soil are also time dependent, when the soil environment is adequate for the activity of *Nitrosomonas* and *Nitrobacter* may lead to a decrease of NH_4^+ and increase of NO_3^- (Brady and Weil, 2002).

A trial was conducted to compare the field extractions with conventional sampling and handling procedures and showed considerable differences in ammonium and nitrate results.

4.1.1 Objectives

The aim of this study was to establish an appropriate sample handling procedure for determination of nitrate (NO_3^-) and ammonium (NH_4^+) speciation in sewage sludge amended soils.

To fulfill this objective three sample handling strategies were tested, there are as follows:

i. Direct Field Extraction- DFE
ii. Field Drying and Extraction - FDE
iii. Laboratory Drying and Extraction - LDE

4.2 Material and methods

4.2.1 Material

A. Amended soil samples

Samples were collected from an existing crop trial at Harbeesfontein, a waste water treatment facility of East Rand Water Company near Kempton Park, Gauteng province, South Africa, with sandy clay loam soils that have received 16 ton per ha of activated anaerobically digested sewage sludge. Distributed in two seasons, half (8 t ha^{-1}) was applied in winter season and another half in summer season of the year 2005. Samples were taken in triplicate.

B. Equipment

Plastic bottles with 200 cm^3 capacity, plastic cup with 10 cm^3 capacity, aluminum foil tart plate folder, weighing scale, horizontal shaking, filtration stand, fridge, kjeldhal distillation apparatus, and titration system.

C. Chemicals

Potassium chloride (KCl) 1 M solution, Sodium hydroxide (NaOH) 12.5M, Devarda's alloy powder, Hydrochloric acid (HCl) 0.01M, ethanol (C_2H_5OH), Boric acid (H_3BO_3) and indicator (methyl blue and methyl red).

4.2.2 Methods

A. Sample handling strategies

Three sample handling strategies were tested in samples collected from the plots that have received 16 ton of sewage sludge. There are as follow:

- Direct Field Extraction (DFE) – samples were collected and immediately extracted in the field, then taken to the laboratory for analysis.
- Field Drying and Extraction (FDE) – samples were collected, air dried immediately, sieved and extracted in the field, then taken to the laboratory for analysis.
- Laboratory Drying Extraction (LDE) – samples were collected, taken to the laboratory air dried for 24 hours sieved, extracted and then analysed.

i) Direct Field Extraction

A known volume of instant collected soil samples that passed through a 2 mm sieve, were directly transferred to containers with 100 cm^3 2 *M* KCl solution to extract the extractable and exchangeable ammonium and nitrate and taken to the laboratory within few hours for subsequent analysis.

ii) Field Drying and Extraction

The collected soil samples were spread out in aluminum foil tart plate left to dry in the field. Afterwards the samples were sieved through a 2 mm sieve then a known volume of these air dried soil was directly transferred to containers with 100 cm^3 2 *M* KCl solution to extract the extractable and exchangeable ammonium and nitrate and taken to the laboratory for subsequent ammonium and nitrate determinations.

iii) Laboratory Drying and Extraction

Collected samples were taken to the laboratory air dried for 24 hours at room temperature (~ 22 – 25 ^oC), sieved to pass a 2 mm sieve, 10 g of soil were extracted in 100 cm^3 of 2 M KCl solution and ammonium and nitrate were determined.

NB.1 For field extractions **DFE** and **FDE** the used plastic cups for measuring the soil volume were filled with respective soil samples taken to the laboratory weighed and then oven dried and weighed again to obtain the mass of soil extracted in dry mass basis, for the final calculations.

NB.2 If there is time constraint after filtration extracts should be kept in the fridge. At the moment of determination samples are taken out from the fridge left to reach room temperature and then carry out the analysis.

B. Extractable and exchangeable NH_4^+ and NO_3^- determinations

Sample extracts from all handling strategies were kept in the fridge, while waiting to be analysed, due to time constraint, to stop any possible conversion of N-forms. The temperature in the fridge was at 1.9 °C, sufficient to prevent any microbial activity. At the moment of determination samples were taken out from the fridge left until they reached room temperature then NH_4^+ and NO_3^- determined.

Procedure

50 ml of soil extracts was transferred into a distil tube and 20 ml of a 12.5*M* NaOH solution was added (sufficient amount to convert all NH_4^+ present in the soil extract into NH_4OH) and distilled through micro- Kjeldahl steam distil system. The NH_3 formed was collected in mixture solution of H_3BO_3-indicator with purple colour. Ammonia entering in this solution formed a green complex with boric acid- indicator and then titrated with HCl 0.01*M* until the green colour changes. At the end point of titration volume of hydrochloric acid was recorded. In the remained extract on the Kjeldahl distil tube approximately 2 mg of devarda's alloy powder was added after cooling to reduce all NO_3^- and NO_2^- into NH_4^+, which in turn react with the excess of NaOH and distilled again following the same procedure, and the volume of hydrochloric acid was recorded. The first step of distillation was for ammonium determination and the second for nitrate.

C. Extractable and exchangeable NH_4^+ and NO_3^- calculation

The concentration of NH_4^+ and NO_3^- in the samples was obtained based on the equi-molar displacement reaction principle as explained in chapter III– 3.2.6.1 (equation 8).

4.3 Results and discussion

4.3.1 Sample handling effect on NH_4^+ and NO_3^- content in sewage sludge amended soil

Under dry land condition the content of soil NH_4^+ speciation showed a little increase along the drying process. It was almost the same for all sample handling strategies whilst for NO_3^- content an increase was observed from Direct Field Extraction to Laboratory Drying and Extraction (Fig. 4.1). This could be explained by the fact that the organic N mineralizes first into ammonium, which in turn at well aerated conditions is converted into nitrate through nitrifying bacteria activity. Results are in agreement with findings of (Ashok *et al.*, 2006) who reported an increase in NH_4^+concentration during the first fifteen days of incubation on sewage sludge amended soil followed by a sharp decline indicating rapid transformation into NO_3^-.

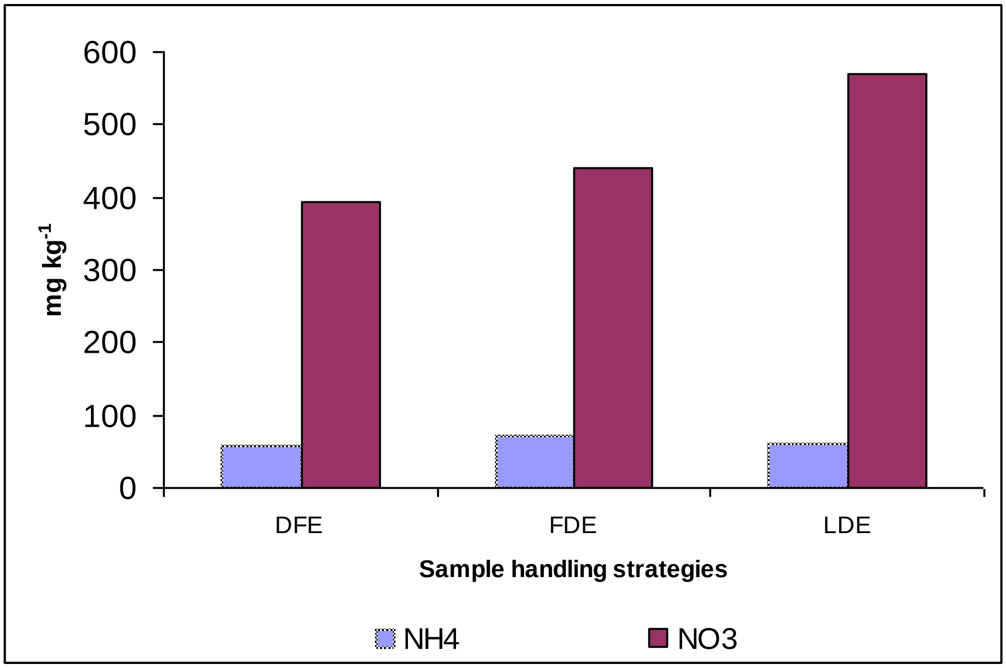

Figure 4.1 Concentration of NH_4^+ and NO_3^- versus sample handling procedures

4.3.2 Statistical analysis

The general linear model procedure for SAS program and the Tukey's test grouping were used. Based on the ANOVA tables sample handling procedures were significantly different. Difference between DFE and FDE was highly significant for ammonium, while in relation to nitrate content these strategies were not statistically different. The effects of DFE and LDE relative to ammonium content were not significantly different however their effects on nitrate content were statistically different at 5 % of significance level. Effects of FDE and LDE were significantly different for both ammonium and nitrate content (Table 4.1).

Table 4.1 Levels of significance for sample handling strategies

Sampling strategy	**FDE**		**LDE**	
	NH_4^+	**NO_3^-**	**NH_4^+**	**NO_3^-**
DFE	0.0008 **	0.3593 *ns*	0.2191 ns	0.0103*
FDE	-	-	0.003 **	0.036 *

ns *= Not significant ($p > 0.05$);* ***** *Significant at α=5% ($p < 0.05$);* ****** *Highly significant α=1% ($p < 0.01$)*

From Table 4.2, ammonium content obtained with DFE was not significantly different from that of the LDE, The reason is that mineralized NH_4^+ was being simultaneously converted into NO_3^- and replaced by mineralization of organic N from the system soil/sewage sludge. However the ammonium content for FDE was significantly higher compared to DFE and LDE. The reason could be that drying in an open sun increased mineralization as a result of temperature effect. On the other hand the shortest time from collection-drying and extraction did not give chance for nitrifying bacteria to perform. The nitrate content for DFE and for FDE samples was significantly lower from that of the LDE samples as a result of mineralization and nitrification processes occurring along the period going from sample collection, drying up until the sample extraction in the laboratory.

Table 4.2 Ranking and treatment mean comparison

Treatment	**NH_4^+** [mg kg^{-1}]	**t grouping**	**Treatment**	**NO_3^-** [mg kg^{-1}]	**t grouping**
FDE	70.42	a	**LDE**	568.8	a
LDE	59.54	b	**FDE**	441.1	b
DFE	56.43	b	**DFE**	393.9	b

**Treatment means followed with the same letter are not statistically different at = 5% ($p < 0.05$)*

4.4 Conclusions and recommendations

4.4.1 Conclusions

The sample handling strategy had greater influence on determined soil nitrate and ammonium content. Artifacts introduced by drying result in an overestimation of nitrification as well as mineralization in the sewage sludge amended soils. The evidences were sufficient to conclude that the Direct Field Extraction strategy was more adequate to figure the soil NH_4^+ and NO_3^- content at the point in time of sampling.

The results agreed with Franzen and Cihacek (2004) findings who concluded that samples intended for NO_3-N determination should be transported immediately to a soil testing laboratory in a cold ice chest or air-dried immediately after collection and then taken for immediate laboratory analysis to prevent alteration of N concentrations through microbial activity.

4.4.2 Recommendations

When developing models to assess nitrate pollution risk and in soil fertility management for an effective use of sewage sludge nitrogen, the Direct Field Extraction strategy is recommended

Concurrently to application of DFE strategy, samples should be collected closer to planting time to minimize the gap between the determined soil nitrates NO_3^- content with the availability of N at a point in time of sampling.

4.5 References

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5. APPENDICE

A_1. Statistical analysis for temperature and water potential effect on net N release

A$_{1.1}$

```
Stable ‘Vlakplaas’ sewage sludge amended soil    10:45 Monday, March 19, 2007   3

                    Obs    Temp    Moist    rep      V1

                      1     T1      W1       1     2.681
                      2     T1      W1       2     2.912
                      3     T1      W1       3     2.713
                      4     T1      W2       1     2.075
                      5     T1      W2       2     2.063
                      6     T1      W2       3     1.944
                      7     T1      W3       1     3.224
                      8     T1      W3       2     3.463
                      9     T1      W3       3     3.403
                     10     T2      W1       1     2.700
                     11     T2      W1       2     3.106
                     12     T2      W1       3     2.883
                     13     T2      W2       1     2.477
                     14     T2      W2       2     2.421
                     15     T2      W2       3     2.489
                     16     T2      W3       1     2.211
                     17     T2      W3       2     2.282
                     18     T2      W3       3     2.262
                     19     T3      W1       1     3.213
                     20     T3      W1       2     3.332
                     21     T3      W1       3     3.214
                     22     T3      W2       1     3.069
                     23     T3      W2       2     3.757
                     24     T3      W2       3     3.058
                     25     T3      W3       1     3.528
                     26     T3      W3       2     3.651
                     27     T3      W3       3     3.324


         Stable sewage sludge ‘Vlakplaas’     10:45 Monday, March 19, 2007   4

                              The GLM Procedure

                           Class Level Information

                       Class          Levels    Values

                       Temp                3    T1 T2 T3

                       Moist               3    W1 W2 W3

                       Rep                 3    1 2 3


                   Number of Observations Read          27
                   Number of Observations Used          27
```

```
Stable sewage sludge ‘Vlakplaas’     10:45 Monday, March 19, 2007   5

                                 The GLM Procedure

Dependent Variable: V1   Total mineralized N

                                             Sum of
       Source                      DF        Squares     Mean Square    F Value    Pr > F

       Model                        8     6.71439496      0.83929937      27.70    <.0001

       Error                       18     0.54534667      0.03029704

       Corrected Total             26     7.25974163


                      R-Square     Coeff Var      Root MSE       V1 Mean

                      0.924881      6.067564      0.174060      2.868704


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

       Temp                         2     3.27215030      1.63607515      54.00    <.0001
       Moist                        2     1.03258007      0.51629004      17.04    <.0001
       Temp*Moist                   4     2.40966459      0.60241615      19.88    <.0001


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

       Temp                         2     3.27215030      1.63607515      54.00    <.0001
       Moist                        2     1.03258007      0.51629004      17.04    <.0001
       Temp*Moist                   4     2.40966459      0.60241615      19.88    <.0001

                   Stable sewage sludge ‘Vlakplaas’     10:45 Monday, March 19, 2007   6

                                 The GLM Procedure

                     Tukey's Studentized Range (HSD) Test for V1   #

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


                        Alpha                                 0.05
                        Error Degrees of Freedom                18
                        Error Mean Square                 0.030297
                        Critical Value of Studentized Range  3.60930
                        Minimum Significant Difference      0.2094





Means with the same letter are not significantly different.


                    Tukey Grouping          Mean      N    Temp

                                 A       3.34956      9    T3

                                 B       2.71978      9    T1

                                 B       2.53678      9    T2
```

```
      Stable sewage sludge ‘Vlakplaas’     10:45 Monday, March 19, 2007   7

                              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                  18
                  Error Mean Square                   0.030297
                  Critical Value of Studentized Range  3.60930
                  Minimum Significant Difference        0.2094


               Means with the same letter are not significantly different.

                Tukey Grouping          Mean      N    Moist

                             A       3.03867      9    W3

                             A       2.97267      9    W1

                             B       2.59478      9    W2

               Stable sewage sludge ‘Vlakplaas’     10:45 Monday, March 19, 2007   8

                              The GLM Procedure


               Level of     Level of           --------------V1-------------
               Temp         Moist        N             Mean          Std Dev

               T1           W1           3       2.76866667       0.12515723
               T1           W2           3       2.02733333       0.07241777
               T1           W3           3       3.36333333       0.12433959
               T2           W1           3       2.89633333       0.20332814
               T2           W2           3       2.46233333       0.03629509
               T2           W3           3       2.25166667       0.03661056
               T3           W1           3       3.25300000       0.06841783
               T3           W2           3       3.29466667       0.40043019
               T3           W3           3       3.50100000       0.16516356


               Stable sewage sludge ‘Vlakplaas’     10:45 Monday, March 19, 2007   9

                              The GLM Procedure
                             Least Squares Means

                                       Standard                      LSMEAN
               Temp        V1 LSMEAN          Error     Pr > |t|       Number

               T1         2.71977778      0.05802015      <.0001            1
               T2         2.53677778      0.05802015      <.0001            2
               T3         3.34955556      0.05802015      <.0001            3

                        Least Squares Means for effect Temp
                         Pr > |t| for H0: LSMean(i)=LSMean(j)

                              Dependent Variable: V1

                  i/j              1              2              3

                    1                        0.0387         <.0001
                    2         0.0387                        <.0001
                    3         <.0001         <.0001
```

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


                                              Standard                  LSMEAN
                  Moist       V1 LSMEAN          Error    Pr > |t|      Number

                  W1         2.97266667     0.05802015      <.0001           1
                  W2         2.59477778     0.05802015      <.0001           2
                  W3         3.03866667     0.05802015      <.0001           3


                              Least Squares Means for effect Moist
                              Pr > |t| for H0: LSMean(i)=LSMean(j)

                                    Dependent Variable: V1

                         i/j             1             2             3

                            1                     0.0002        0.4317
                            2       0.0002                      <.0001
                            3       0.4317        <.0001


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

```
                   Stable sewage sludge ‘Vlakplaas’    10:45 Monday, March 19, 2007  10

                                        The GLM Procedure
                                      Least Squares Means

                                                  Standard                  LSMEAN
              Temp    Moist      V1 LSMEAN           Error    Pr > |t|      Number

              T1      W1        2.76866667      0.10049384      <.0001           1
              T1      W2        2.02733333      0.10049384      <.0001           2
              T1      W3        3.36333333      0.10049384      <.0001           3
              T2      W1        2.89633333      0.10049384      <.0001           4
              T2      W2        2.46233333      0.10049384      <.0001           5
              T2      W3        2.25166667      0.10049384      <.0001           6
              T3      W1        3.25300000      0.10049384      <.0001           7
              T3      W2        3.29466667      0.10049384      <.0001           8
              T3      W3        3.50100000      0.10049384      <.0001           9


                           Least Squares Means for effect Temp*Moist
                              Pr > |t| for H0: LSMean(i)=LSMean(j)

                                    Dependent Variable: V1

 i/j          1         2        3         4         5         6         7        8         9

    1              <.0001   0.0006    0.3809    0.0449    0.0019    0.0031   0.0016    <.0001
    2    <.0001             <.0001    <.0001    0.0067    0.1319    <.0001   <.0001    <.0001
    3    0.0006    <.0001             0.0041    <.0001    <.0001    0.4476   0.6348    0.3455
    4    0.3809    <.0001   0.0041              0.0068    0.0003    0.0219   0.0118    0.0005
    5    0.0449    0.0067   <.0001    0.0068              0.1556    <.0001   <.0001    <.0001
    6    0.0019    0.1319   <.0001    0.0003    0.1556              <.0001   <.0001    <.0001
    7    0.0031    <.0001   0.4476    0.0219    <.0001    <.0001             0.7727    0.0980
    8    0.0016    <.0001   0.6348    0.0118    <.0001    <.0001    0.7727             0.1638
    9    <.0001    <.0001   0.3455    0.0005    <.0001    <.0001    0.0980   0.1638
```

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

A$_{1.2}$

```
Unstable 'Olifantsfontein' sewage sludge amended soils   09:16 Monday, March 19, 2007 1

                        Obs    Temp    Moist    rep      V1

                          1     T1      W1       1      6.015
                          2     T1      W1       2      6.493
                          3     T1      W1       3      6.293
                          4     T1      W2       1      5.816
                          5     T1      W2       2      5.975
                          6     T1      W2       3      5.967
                          7     T1      W3       1      5.760
                          8     T1      W3       2      5.696
                          9     T1      W3       3      5.696
                         10     T2      W1       1      5.277
                         11     T2      W1       2      6.440
                         12     T2      W1       3      5.551
                         13     T2      W2       1      5.756
                         14     T2      W2       2      5.871
                         15     T2      W2       3      5.855
                         16     T2      W3       1      7.548
                         17     T2      W3       2      7.129
                         18     T2      W3       3      7.305
                         19     T3      W1       1      7.239
                         20     T3      W1       2      7.597
                         21     T3      W1       3      7.490
                         22     T3      W2       1      8.870
                         23     T3      W2       2      9.268
                         24     T3      W2       3      9.427
                         25     T3      W3       1     10.581
                         26     T3      W3       2     10.859
                         27     T3      W3       3      9.984


Unstable sewage sludge 'Olifantsfontein'    09:16 Monday, March 19, 2007         2

                             The GLM Procedure

                          Class Level Information

                      Class         Levels    Values

                      Temp               3    T1 T2 T3
```

```
   Moist             3    W1 W2 W3

   Rep               3    1 2 3


Number of Observations Read          27
Number of Observations Used          27
```

```
Unstable sewage sludge ‘Olifantsfontein’      09:16 Monday, March 19, 2007            3

                              The GLM Procedure

Dependent Variable: V1   net nitrogen mineralized

                                        Sum of
        Source                DF       Squares     Mean Square    F Value    Pr > F

        Model                  8    70.03128141     8.75391018      98.38    <.0001

        Error                 18     1.60160200     0.08897789

        Corrected Total       26    71.63288341


                     R-Square     Coeff Var      Root MSE       V1 Mean

                     0.977642      4.200020      0.298292      7.102148


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

        Temp                   2    50.94189430    25.47094715     286.26    <.0001
        Moist                  2     8.42576585     4.21288293      47.35    <.0001
        Temp*Moist             4    10.66362126     2.66590531      29.96    <.0001


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

        Temp                   2    50.94189430    25.47094715     286.26    <.0001
        Moist                  2     8.42576585     4.21288293      47.35    <.0001
        Temp*Moist             4    10.66362126     2.66590531      29.96    <.0001
```

```
             Unstable sewage sludge ‘Olifantsfontein’   09:16 Monday, March 19, 2007        4

                                  The GLM Procedure

                      Tukey's Studentized Range (HSD) Test for V1

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


                         Alpha                                  0.05
                         Error Degrees of Freedom                 18
                         Error Mean Square                  0.088978
                         Critical Value of Studentized Range  3.60930
                         Minimum Significant Difference       0.3589



 Means with the same letter are not significantly different.


                  Tukey Grouping          Mean       N     Temp

                               A        9.0350       9     T3

                               B        6.3036       9     T2

                               B        5.9679       9     T1
```

```
             Unstable sewage sludge ‘Olifantsfontein’   09:16 Monday, March 19, 2007         5

                                       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                  18
                         Error Mean Square                   0.088978
                         Critical Value of Studentized Range  3.60930
                         Minimum Significant Difference        0.3589


                  Means with the same letter are not significantly different.


                   Tukey Grouping          Mean       N    Moist

                                A        7.8398       9    W3

                                B        6.9783       9    W2

                                C        6.4883       9    W1

             Unstable sewage sludge ‘olifantsfontein’   09:16 Monday, March 19, 2007         6

                                       The GLM Procedure


                 Level of     Level of            --------------V1-------------
                 Temp         Moist         N             Mean          Std Dev

                 T1           W1            3        6.2670000       0.24005833
                 T1           W2            3        5.9193333       0.08957864
                 T1           W3            3        5.7173333       0.03695042
                 T2           W1            3        5.7560000       0.60799753
                 T2           W2            3        5.8273333       0.06229232
                 T2           W3            3        7.3273333       0.21039091
                 T3           W1            3        7.4420000       0.18376343
                 T3           W2            3        9.1883333       0.28691869
                 T3           W3            3       10.4746667       0.44708649

             Unstable sewage sludge ‘Olifantsfontein’   09:16 Monday, March 19, 2007         7


                                       The GLM Procedure
                                      Least Squares Means

                                             Standard                       LSMEAN
                 Temp        V1 LSMEAN          Error    Pr > |t|          Number

                 T1         5.96788889     0.09943054      <.0001               1
                 T2         6.30355556     0.09943054      <.0001               2
                 T3         9.03500000     0.09943054      <.0001               3




                                Least Squares Means for effect Temp
                                Pr > |t| for H0: LSMean(i)=LSMean(j)

                                      Dependent Variable: V1

                      i/j              1              2              3
```

```
                           1              0.0282        <.0001
                           2        0.0282              <.0001
                           3        <.0001        <.0001


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


                                              Standard                 LSMEAN
                  Moist      V1 LSMEAN           Error    Pr > |t|     Number

                  W1        6.48833333      0.09943054      <.0001          1
                  W2        6.97833333      0.09943054      <.0001          2
                  W3        7.83977778      0.09943054      <.0001          3


                             Least Squares Means for effect Moist
                             Pr > |t| for H0: LSMean(i)=LSMean(j)

                                    Dependent Variable: V1

                         i/j             1             2             3

                           1              0.0026        <.0001
                           2        0.0026              <.0001
                           3        <.0001        <.0001



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


Unstable sewage sludge ‘Olifantsfontein’   09:16 Monday, March 19, 2007           8

                                       The GLM Procedure
                                      Least Squares Means

                                                  Standard                 LSMEAN
              Temp    Moist      V1 LSMEAN           Error    Pr > |t|     Number

              T1      W1         6.2670000       0.1722187      <.0001          1
              T1      W2         5.9193333       0.1722187      <.0001          2
              T1      W3         5.7173333       0.1722187      <.0001          3
              T2      W1         5.7560000       0.1722187      <.0001          4
              T2      W2         5.8273333       0.1722187      <.0001          5
              T2      W3         7.3273333       0.1722187      <.0001          6
              T3      W1         7.4420000       0.1722187      <.0001          7
              T3      W2         9.1883333       0.1722187      <.0001          8
              T3      W3        10.4746667       0.1722187      <.0001          9


                          Least Squares Means for effect Temp*Moist
                             Pr > |t| for H0: LSMean(i)=LSMean(j)

                                    Dependent Variable: V1

 i/j          1         2         3         4         5         6         7         8         9

    1              0.1706    0.0367    0.0503    0.0878    0.0004    0.0001    <.0001    <.0001
    2    0.1706              0.4178    0.5110    0.7100    <.0001    <.0001    <.0001    <.0001
    3    0.0367    0.4178              0.8756    0.6569    <.0001    <.0001    <.0001    <.0001
    4    0.0503    0.5110    0.8756              0.7730    <.0001    <.0001    <.0001    <.0001
    5    0.0878    0.7100    0.6569    0.7730              <.0001    <.0001    <.0001    <.0001
    6    0.0004    <.0001    <.0001    <.0001    <.0001              0.6434    <.0001    <.0001
    7    0.0001    <.0001    <.0001    <.0001    <.0001    0.6434              <.0001    <.0001
    8    <.0001    <.0001    <.0001    <.0001    <.0001    <.0001    <.0001              <.0001
```

```
    9    <.0001    <.0001    <.0001    <.0001    <.0001    <.0001    <.0001    <.0001

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

A$_{1.3}$

```
    Unstable ‘Sasol’ sludge amended soil         14:05 Friday, March 19, 2007   1

                         Obs    Temp    Moist    rep     V1

                           1     T1      W1       1     6.6
                           2     T1      W1       2     7.0
                           3     T1      W1       3     6.9
                           4     T1      W2       1     6.4
                           5     T1      W2       2     6.5
                           6     T1      W2       3     6.6
                           7     T1      W3       1     4.8
                           8     T1      W3       2     5.2
                           9     T1      W3       3     5.0
                          10     T2      W1       1     9.7
                          11     T2      W1       2     9.0
                          12     T2      W1       3     9.6
                          13     T2      W2       1     6.8
                          14     T2      W2       2     7.2
                          15     T2      W2       3     7.3
                          16     T2      W3       1     7.0
                          17     T2      W3       2     6.9
                          18     T2      W3       3     7.4
                          19     T3      W1       1     5.5
                          20     T3      W1       2     5.7
                          21     T3      W1       3     5.7
                          22     T3      W2       1     6.2
                          23     T3      W2       2     6.2
                          24     T3      W2       3     6.0
                          25     T3      W3       1     7.2
                          26     T3      W3       2     7.4
                          27     T3      W3       3     7.3

                 Unstable sludge ‘Sasol’        14:05 Friday, March 19, 2007   2

                                 The GLM Procedure

                              Class Level Information

                         Class         Levels    Values

                         Temp               3    T1 T2 T3

                         Moist              3    W1 W2 W3

                         Rep                3    1 2 3
```

```
                                   Number of observations    27




Unstable sludge ‘Sasol’        14:05 Friday, March 19, 2007   3

                                      The GLM Procedure

Dependent Variable: V1   total mineralized N

                                                 Sum of
        Source                      DF          Squares     Mean Square    F Value    Pr > F

        Model                        8      37.49407407      4.68675926     102.05    <.0001

        Error                       18       0.82666667      0.04592593

        Corrected Total             26      38.32074074

                        R-Square     Coeff Var      Root MSE       V1 Mean

                        0.978428      3.160126      0.214303      6.781481


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

        Temp                         2      16.49407407      8.24703704     179.57    <.0001
        Moist                        2       3.68518519      1.84259259      40.12    <.0001
        Temp*Moist                   4      17.31481481      4.32870370      94.25    <.0001

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

        Temp                         2      16.49407407      8.24703704     179.57    <.0001
        Moist                        2       3.68518519      1.84259259      40.12    <.0001
        Temp*Moist                   4      17.31481481      4.32870370      94.25    <.0001


 Unstable sludge ‘Sasol’        14:05 Friday, March 19, 2007   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                  18
                            Error Mean Square                   0.045926
                            Critical Value of Studentized Range  3.60930
                            Minimum Significant Difference        0.2578



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

```
                      Tukey Grouping          Mean         N    Temp

                                   A        7.8778         9    T2

                                   B        6.3556         9    T3

                                   B        6.1111         9    T1

                      Unstable sludge ‘Sasol’        14:05 Friday, March 19, 2007   5

                                         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                  18
                            Error Mean Square                   0.045926
                            Critical Value of Studentized Range  3.60930
                            Minimum Significant Difference        0.2578


                    Means with the same letter are not significantly different.


                     Tukey Grouping          Mean         N    Moist

                                  A        7.3000         9    W1

                                  B        6.5778         9    W2

                                  B        6.4667         9    W3


Unstable sludge ‘Sasol’        14:05 Friday, March 19, 2007   6

                                         The GLM Procedure

                   Level of     Level of              --------------V1-------------
                   Temp         Moist          N                 Mean          Std Dev

                   T1           W1             3       6.83333333       0.20816660
                   T1           W2             3       6.50000000       0.10000000
                   T1           W3             3       5.00000000       0.20000000
                   T2           W1             3       9.43333333       0.37859389
                   T2           W2             3       7.10000000       0.26457513
                   T2           W3             3       7.10000000       0.26457513
                   T3           W1             3       5.63333333       0.11547005
                   T3           W2             3       6.13333333       0.11547005
                   T3           W3             3       7.30000000       0.10000000

Unstable sewage sludge ‘Sasol’        14:05 Friday, March 19, 2007   7

                                         The GLM Procedure
                                        Least Squares Means

                                                Standard                  LSMEAN
                    Temp       V1 LSMEAN           Error    Pr > |t|      Number

                    T1        6.11111111      0.07143445      <.0001           1
                    T2        7.87777778      0.07143445      <.0001           2
                    T3        6.35555556      0.07143445      <.0001           3
```

```
                         Least Squares Means for effect Temp
                         Pr > |t| for H0: LSMean(i)=LSMean(j)

                                Dependent Variable: V1

                    i/j             1             2             3

                      1                        <.0001        0.0263
                      2        <.0001                        <.0001
                      3        0.0263          <.0001


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


                                         Standard                  LSMEAN
               Moist        V1 LSMEAN       Error    Pr > |t|      Number

               W1          7.30000000  0.07143445     <.0001            1
               W2          6.57777778  0.07143445     <.0001            2
               W3          6.46666667  0.07143445     <.0001            3


                      Least Squares Means for effect Moist
                         Pr > |t| for H0: LSMean(i)=LSMean(j)

                                Dependent Variable: V1

                    i/j             1             2             3

                      1                        <.0001        <.0001
                      2        <.0001                        0.2859
                      3        <.0001          0.2859


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


                 Unstable sludge 'Sasol'        14:05 Friday, March 19, 2007   8

                                  The GLM Procedure
                                 Least Squares Means

                                             Standard                  LSMEAN
           Temp    Moist        V1 LSMEAN       Error    Pr > |t|      Number

           T1      W1          6.83333333  0.12372810      <.0001           1
           T1      W2          6.50000000  0.12372810      <.0001           2
           T1      W3          5.00000000  0.12372810      <.0001           3
           T2      W1          9.43333333  0.12372810      <.0001           4
           T2      W2          7.10000000  0.12372810      <.0001           5
           T2      W3          7.10000000  0.12372810      <.0001           6
           T3      W1          5.63333333  0.12372810      <.0001           7
           T3      W2          6.13333333  0.12372810      <.0001           8
           T3      W3          7.30000000  0.12372810      <.0001           9

                      Least Squares Means for effect Temp*Moist
                         Pr > |t| for H0: LSMean(i)=LSMean(j)

                                Dependent Variable: V1

  i/j         1         2         3         4         5         6         7         8         9

     1               0.0729    <.0001    <.0001    0.1449    0.1449    <.0001    0.0008    0.0157
```

```
      2    0.0729              <.0001    <.0001    0.0030    0.0030    0.0001    0.0505    0.0002
      3    <.0001    <.0001              <.0001    <.0001    <.0001    0.0020    <.0001    <.0001
      4    <.0001    <.0001    <.0001              <.0001    <.0001    <.0001    <.0001    <.0001
      5    0.1449    0.0030    <.0001    <.0001              1.0000    <.0001    <.0001    0.2680
      6    0.1449    0.0030    <.0001    <.0001    1.0000              <.0001    <.0001    0.2680
      7    <.0001    0.0001    0.0020    <.0001    <.0001    <.0001              0.0105    <.0001
      8    0.0008    0.0505    <.0001    <.0001    <.0001    <.0001    0.0105              <.0001
      9    0.0157    0.0002    <.0001    <.0001    0.2680    0.2680    <.0001    <.0001


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

A$_2$ Statistical analysis for temperature and water potential on NH_4^+ and NO_3^-

A$_{2.1}$ Stable ‘Vlakplaas’ sewage sludge amended soil

```
A 2.1.1 Effect of temperature and moist on NH4 release  08:00 Sunday, November 28, 2008       1

                                   Obs    Temp    Mois    Rep      VI

                                     1     T1      W1      1     0.650
                                     2     T1      W1      2     0.602
                                     3     T1      W1      3     0.554
                                     4     T1      W2      1     0.671
                                     5     T1      W2      2     0.838
                                     6     T1      W2      3     0.599
                                     7     T1      W3      1     0.822
                                     8     T1      W3      2     0.782
                                     9     T1      W3      3     0.790
                                    10     T2      W1      1     0.066
                                    11     T2      W1      2     0.066
                                    12     T2      W1      3     0.066
                                    13     T2      W2      1     0.033
                                    14     T2      W2      2     0.033
                                    15     T2      W2      3     0.033
                                    16     T2      W3      1     0.113
                                    17     T2      W3      2     0.113
                                    18     T2      W3      3     0.113
                                    19     T3      W1      1     3.115
                                    20     T3      W1      2     2.996
                                    21     T3      W1      3     3.055
                                    22     T3      W2      1     2.958
                                    23     T3      W2      2     3.526
                                    24     T3      W2      3     2.906
                                    25     T3      W3      1     3.324
```

```
                                   26      T3      W3      2      3.249
                                   27      T3      W3      3      3.259


Effect of temperature and moist on NH4 release   08:00 Sunday, November 28, 2008     2


                                   The GLM Procedure

                                Class Level Information

Class        Levels  Values

Temp              3  T1 T2 T3

Mois              3  W1 W2 W3

Rep               3  1 2 3

VI               21  0.033 0.066 0.113 0.554 0.599 0.602 0.65 0.671 0.782 0.79 0.822 0.838 2.906
                     2.958 2.996 3.055 3.115 3.249 3.259 3.324 3.526


                              Number of observations    27

Effect of temperature and moist on NH4 release       08:00 Sunday, November 28, 2008     3


                                   The GLM Procedure

                              Dependent Variable: VI   NH4

                                              Sum of
       Source                      DF         Squares     Mean Square    F Value    Pr > F

       Model                        8     47.91692052      5.98961506     381.58    <.0001

       Error                       18      0.28254200      0.01569678

       Corrected Total             26     48.19946252


                        R-Square     Coeff Var      Root MSE       VI Mean

                        0.994138      9.574163      0.125287      1.308593


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

       Temp                         2     47.77301807     23.88650904    1521.75    <.0001
       Mois                         2      0.11353252      0.05676626       3.62    0.0478
       Temp*Mois                    4      0.03036993      0.00759248       0.48    0.7475


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

       Temp                         2     47.77301807     23.88650904    1521.75    <.0001
       Mois                         2      0.11353252      0.05676626       3.62    0.0478
       Temp*Mois                    4      0.03036993      0.00759248       0.48    0.7475


Effect of temperature and moist on NH4 release     08:00 Sunday, November 28, 2008        4


                                   The GLM Procedure

                         Tukey's Studentized Range (HSD) Test for VI

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                  18
             Error Mean Square                   0.015697
             Critical Value of Studentized Range  3.60930
             Minimum Significant Difference        0.1507



Means with the same letter are not significantly different.


          Tukey Grouping          Mean      N    Temp

                A       3.15422      9    T3

                B       0.70089      9    T1

                C       0.07067      9    T2

Effect of temperature and moist on NH4 release   08:00 Sunday, November 28, 2008         5


                            The GLM Procedure

             Tukey's Studentized Range (HSD) Test for VI

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                  18
             Error Mean Square                   0.015697
             Critical Value of Studentized Range  3.60930
             Minimum Significant Difference        0.1507


         Means with the same letter are not significantly different.


           Tukey Grouping          Mean      N    Mois

                 A       1.39611      9    W3

              B  A       1.28856      9    W2

              B          1.24111      9    W1

Effect of temperature and moist on NH4 release     08:00 Sunday, November 28, 2008    6


                            The GLM Procedure


         Level of     Level of              --------------VI-------------
         Temp         Mois          N              Mean          Std Dev

         T1           W1            3        0.60200000       0.04800000
         T1           W2            3        0.70266667       0.12260642
         T1           W3            3        0.79800000       0.02116601
         T2           W1            3        0.06600000       0.00000000
         T2           W2            3        0.03300000       0.00000000
         T2           W3            3        0.11300000       0.00000000
         T3           W1            3        3.05533333       0.05950070
         T3           W2            3        3.13000000       0.34393023
         T3           W3            3        3.27733333       0.04072264
```

```
Effect of temperature and moist on NH4 release   08:00 Sunday, November 28, 2008        7

                                  The GLM Procedure
                                 Least Squares Means

                                           Standard                      LSMEAN
                   Temp       VI LSMEAN       Error    Pr > |t|        Number

                   T1        0.70088889     0.04176226      <.0001             1
                   T2        0.07066667     0.04176226      0.1079             2
                   T3        3.15422222     0.04176226      <.0001             3



                            Least Squares Means for effect Temp
                         Pr > |t| for H0: LSMean(i)=LSMean(j)

                                 Dependent Variable: VI

                      i/j              1              2             3

                         1                        <.0001        <.0001
                         2        <.0001                        <.0001
                         3        <.0001          <.0001

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

                                           Standard                      LSMEAN
                   Mois       VI LSMEAN       Error    Pr > |t|        Number

                   W1        1.24111111     0.04176226      <.0001             1
                   W2        1.28855556     0.04176226      <.0001             2
                   W3        1.39611111     0.04176226      <.0001             3

                         Least Squares Means for effect Mois
                         Pr > |t| for H0: LSMean(i)=LSMean(j)

                                 Dependent Variable: VI

                      i/j              1              2             3

                         1                        0.4323        0.0172
                         2        0.4323                        0.0853
                         3        0.0172          0.0853

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



Effect of temperature and moist on NH4 release-day 56     08:00 Sunday, November 28, 2008    8

                                  The GLM Procedure
                                 Least Squares Means

                                               Standard                      LSMEAN
                 Temp    Mois        VI LSMEAN       Error    Pr > |t|        Number

                 T1      W1        0.60200000     0.07233436      <.0001             1
                 T1      W2        0.70266667     0.07233436      <.0001             2
```

```
              T1      W3       0.79800000      0.07233436      <.0001           3
              T2      W1       0.06600000      0.07233436      0.3736           4
              T2      W2       0.03300000      0.07233436      0.6537           5
              T2      W3       0.11300000      0.07233436      0.1357           6
              T3      W1       3.05533333      0.07233436      <.0001           7
              T3      W2       3.13000000      0.07233436      <.0001           8
              T3      W3       3.27733333      0.07233436      <.0001           9



                               Least Squares Means for effect Temp*Mois
                                 Pr > |t| for H0: LSMean(i)=LSMean(j)

                                        Dependent Variable: VI

   i/j           1          2          3          4          5          6          7          8          9

     1                 0.3381     0.0714     <.0001     <.0001     0.0001     <.0001     <.0001     <.0001
     2      0.3381                0.3637     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001
     3      0.0714     0.3637                <.0001     <.0001     <.0001     <.0001     <.0001     <.0001
     4      <.0001     <.0001     <.0001                0.7507     0.6514     <.0001     <.0001     <.0001
     5      <.0001     <.0001     <.0001     0.7507                0.4444     <.0001     <.0001     <.0001
     6      0.0001     <.0001     <.0001     0.6514     0.4444                <.0001     <.0001     <.0001
     7      <.0001     <.0001     <.0001     <.0001     <.0001     <.0001                0.4748     0.0436
     8      <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     0.4748                0.1670
     9      <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     0.0436     0.1670

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



Effect of temperature and moist on NH4 release    08:00 Sunday, November 28, 2008        9


                                       The UNIVARIATE Procedure
                                         Variable:  VI  (NH4)

                                               Moments

                 N                          27    Sum Weights                 27
                 Mean               1.30859259    Sum Observations        35.332
                 Std Deviation       1.3615526    Variance            1.85382548
                 Skewness           0.64353633    Kurtosis             -1.491104
                 Uncorrected SS      94.434656    Corrected SS        48.1994625
                 Coeff Variation    104.047097    Std Error Mean      0.26203092



                                     Basic Statistical Measures

                         Location                    Variability

                      Mean     1.308593     Std Deviation            1.36155
                      Median   0.671000     Variance                 1.85383
                      Mode     0.033000     Range                    3.49300
                                            Interquartile Range      2.88300

              NOTE: The mode displayed is the smallest of 3 modes with a count of 3.


                                    Tests for Location: Mu0=0

                        Test           -Statistic-    -----p Value------

                        Student's t    t  4.994039    Pr > |t|    <.0001
```

```
                    Sign           M      13.5    Pr >= |M|   <.0001
                    Signed Rank    S       189    Pr >= |S|   <.0001



                              Tests for Normality

               Test                  --Statistic---    -----p Value------

               Shapiro-Wilk          W     0.765424    Pr < W      <0.0001
               Kolmogorov-Smirnov    D     0.301856    Pr > D      <0.0100
               Cramer-von Mises      W-Sq  0.490143    Pr > W-Sq   <0.0050
               Anderson-Darling      A-Sq  2.769634    Pr > A-Sq   <0.0050


                            Quantiles (Definition 5)

                             Quantile      Estimate

                             100% Max         3.526
                             99%              3.526
                             95%              3.324
                             90%              3.259




Effect of temperature and moist on NH4 release   08:00 Sunday, November 28, 2008       10

                            The UNIVARIATE Procedure
                              Variable:  VI  (NH4)

                            Quantiles (Definition 5)

                             Quantile      Estimate

                             75% Q3           2.996
                             50% Median       0.671
                             25% Q1           0.113
                             10%              0.033
                             5%               0.033
                             1%               0.033
                             0% Min           0.033


                              Extreme Observations

                         -----Lowest----        ----Highest----

                          Value      Obs          Value      Obs

                          0.033       15          3.115       19
                          0.033       14          3.249       26
                          0.033       13          3.259       27
                          0.066       12          3.324       25
                          0.066       11          3.526       23


                               Frequency Counts

                  Percents                          Percents                          Percents
    Value Count  Cell    Cum          Value Count  Cell    Cum          Value Count  Cell    Cum
```

```
0.033     3  11.1  11.1          0.671     1   3.7  51.9          2.996     1   3.7  77.8
0.066     3  11.1  22.2          0.782     1   3.7  55.6          3.055     1   3.7  81.5
0.113     3  11.1  33.3          0.790     1   3.7  59.3          3.115     1   3.7  85.2
0.554     1   3.7  37.0          0.822     1   3.7  63.0          3.249     1   3.7  88.9
0.599     1   3.7  40.7          0.838     1   3.7  66.7          3.259     1   3.7  92.6
0.602     1   3.7  44.4          2.906     1   3.7  70.4          3.324     1   3.7  96.3
0.650     1   3.7  48.1          2.958     1   3.7  74.1          3.526     1   3.7 100.0
```

Effect of temperature and moist on NH_4 release   08:00 Sunday, November 28, 2008   11

```
              The UNIVARIATE Procedure
                Variable:  VI (NH4)

  Stem Leaf                     #            Boxplot
     3 5                        1               |
     3 0011233                  7            +-----+
     2 9                        1            |     |
     2                                       |     |
     1                                       |     |
     1                                       |  +  |
     0 666678888                9            *-----*
     0 000111111                9            +-----+
       ----+----+----+----+


                   Normal Probability Plot
3.75+                                         +++*
    |                                 * ** *++*
    |                             *** ++++
    |                              +++
    |                          ++++
    |                      ++++
    |                    +*********
0.25+     *   *  * * +**+*
     +----+----+----+----+----+----+----+----+----+----+
         -2        -1         0        +1        +2
```

A 2.1.2 Effect of temperature and moist on NO_3 release   08:50 Sunday, November 28, 2008   1

```
Obs    Temp    Mois    Rep       VI

  1     T1      W1      1     2.554
  2     T1      W1      2     2.634
  3     T1      W1      3     2.602
  4     T1      W2      1     2.055
  5     T1      W2      2     1.956
  6     T1      W2      3     1.964
  7     T1      W3      1     2.912
  8     T1      W3      2     3.031
  9     T1      W3      3     3.043
 10     T2      W1      1     2.944
 11     T2      W1      2     2.992
 12     T2      W1      3     2.864
 13     T2      W2      1     2.642
 14     T2      W2      2     2.602
 15     T2      W2      3     2.638
 16     T2      W3      1     2.410
 17     T2      W3      2     2.609
 18     T2      W3      3     2.530
 19     T3      W1      1     0.095
 20     T3      W1      2     0.334
 21     T3      W1      3     0.164
 22     T3      W2      1     0.151
 23     T3      W2      2     0.191
 24     T3      W2      3     0.151
 25     T3      W3      1     0.273
 26     T3      W3      2     0.193
 27     T3      W3      3     0.205
```

```
Effect of temperature and moist on NO3        08:50 Sunday, November 28, 2008      2


                                         The GLM Procedure

                                      Class Level Information

Class       Levels  Values

Temp             3  T1 T2 T3

Mois             3  W1 W2 W3

Rep              3  1 2 3

VI              25  0.095 0.151 0.164 0.191 0.193 0.205 0.273 0.334 1.956 1.964 2.055 2.41 2.53
                    2.554 2.602 2.609 2.634 2.638 2.642 2.864 2.912 2.944 2.992 3.031 3.043

                        Number of observations    27


Effect of temperature and moist on NO3 release     08:50 Sunday, November 28, 2008     3


                                          The GLM Procedure

                                    Dependent Variable: VI   NO3

                                                Sum of
       Source                      DF          Squares     Mean Square    F Value    Pr > F

       Model                        8      36.92927941      4.61615993     986.31    <.0001

       Error                       18       0.08424400      0.00468022

       Corrected Total             26      37.01352341


                        R-Square     Coeff Var      Root MSE       VI Mean

                        0.997724      3.789836      0.068412      1.805148


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

       Temp                         2      35.11181896     17.55590948    3751.08    <.0001
       Mois                         2       0.59937607      0.29968804      64.03    <.0001
       Temp*Mois                    4       1.21808437      0.30452109      65.07    <.0001


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

       Temp                         2      35.11181896     17.55590948    3751.08    <.0001
       Mois                         2       0.59937607      0.29968804      64.03    <.0001
       Temp*Mois                    4       1.21808437      0.30452109      65.07    <.0001
Effect of temperature and moist on NO3 release 08:50 Sunday, November 28, 2008

                                          The GLM Procedure

                             Tukey's Studentized Range (HSD) Test for VI

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                18
                      Error Mean Square                  0.00468
                      Critical Value of Studentized Range  3.60930
                      Minimum Significant Difference      0.0823


Means with the same letter are not significantly different.


                 Tukey Grouping          Mean      N    Temp

                              A       2.69233      9    T2

                              B       2.52789      9    T1

                              C       0.19522      9    T3

Effect of temperature and moist on NO3 release    08:50 Sunday, November 28, 2008       5

                                 The GLM Procedure

                      Tukey's Studentized Range (HSD) Test for VI

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                18
                      Error Mean Square                  0.00468
                      Critical Value of Studentized Range  3.60930
                      Minimum Significant Difference      0.0823


                Means with the same letter are not significantly different.


                 Tukey Grouping          Mean      N    Mois

                              A       1.91178      9    W3

                              A       1.90922      9    W1

                              B       1.59444      9    W2

Effect of temperature and moist on NO3 release     08:50 Sunday, November 28, 2008     6

                                 The GLM Procedure


               Level of     Level of           --------------VI-------------
               Temp         Mois         N            Mean         Std Dev

               T1           W1           3      2.59666667      0.04026578
               T1           W2           3      1.99166667      0.05499394
               T1           W3           3      2.99533333      0.07241777
               T2           W1           3      2.93333333      0.06466323
               T2           W2           3      2.62733333      0.02203028
               T2           W3           3      2.51633333      0.10020146
               T3           W1           3      0.19766667      0.12300542
               T3           W2           3      0.16433333      0.02309401
               T3           W3           3      0.22366667      0.04314317

Effect of temperature and moist on NO3 release   08:50 Sunday, November 28, 2008     7

                                 The GLM Procedure
```

```
                              Least Squares Means

                                          Standard                     LSMEAN
         Temp        VI LSMEAN               Error    Pr > |t|          Number

         T1         2.52788889      0.02280405      <.0001               1
         T2         2.69233333      0.02280405      <.0001               2
         T3         0.19522222      0.02280405      <.0001               3


                          Least Squares Means for effect Temp
                          Pr > |t| for H0: LSMean(i)=LSMean(j)

                                Dependent Variable: VI

                i/j              1             2             3

                   1                      <.0001        <.0001
                   2        <.0001                      <.0001
                   3        <.0001        <.0001


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


                                         Standard                     LSMEAN
         Mois        VI LSMEAN              Error    Pr > |t|          Number

         W1         1.90922222      0.02280405      <.0001               1
         W2         1.59444444      0.02280405      <.0001               2
         W3         1.91177778      0.02280405      <.0001               3

                          Least Squares Means for effect Mois
                          Pr > |t| for H0: LSMean(i)=LSMean(j)

                                Dependent Variable: VI

                i/j              1             2             3

                   1                      <.0001        0.9377
                   2        <.0001                      <.0001
                   3        0.9377        <.0001


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


Effect of temperature and moist on NO3 release          08:50 Sunday, November 28, 2008        8


                               The GLM Procedure
                              Least Squares Means

                                             Standard                     LSMEAN
     Temp     Mois        VI LSMEAN             Error    Pr > |t|          Number

     T1       W1         2.59666667      0.03949777      <.0001               1
     T1       W2         1.99166667      0.03949777      <.0001               2
     T1       W3         2.99533333      0.03949777      <.0001               3
     T2       W1         2.93333333      0.03949777      <.0001               4
     T2       W2         2.62733333      0.03949777      <.0001               5
     T2       W3         2.51633333      0.03949777      <.0001               6
     T3       W1         0.19766667      0.03949777      <.0001               7
     T3       W2         0.16433333      0.03949777      0.0006               8
     T3       W3         0.22366667      0.03949777      <.0001               9
```

```
                              Least Squares Means for effect Temp*Mois
                                Pr > |t| for H0: LSMean(i)=LSMean(j)

                                     Dependent Variable: VI

  i/j          1          2          3          4          5          6          7          8          9

     1                <.0001     <.0001     <.0001     0.5897     0.1675     <.0001     <.0001     <.0001
     2     <.0001                <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001
     3     <.0001     <.0001                0.2816     <.0001     <.0001     <.0001     <.0001     <.0001
     4     <.0001     <.0001     0.2816                <.0001     <.0001     <.0001     <.0001     <.0001
     5     0.5897     <.0001     <.0001     <.0001                0.0623     <.0001     <.0001     <.0001
     6     0.1675     <.0001     <.0001     <.0001     0.0623                <.0001     <.0001     <.0001
     7     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001                0.5581     0.6472
     8     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     0.5581                0.3022
     9     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     0.6472     0.3022


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

Effect of temperature and moist on NO3 release-day 56   08:50 Sunday, November 28, 2008     9


                                    The UNIVARIATE Procedure
                                      Variable:  VI  (NO3)

                                             Moments

                 N                          27    Sum Weights                 27
                 Mean               1.80514815    Sum Observations        48.739
                 Std Deviation      1.19314586    Variance            1.42359705
                 Skewness           -0.5870878    Kurtosis            -1.5745519
                 Uncorrected SS     124.994639    Corrected SS        37.0135234
                 Coeff Variation     66.096839    Std Error Mean      0.22962103



                                   Basic Statistical Measures

                       Location                    Variability

                   Mean     1.805148     Std Deviation            1.19315
                   Median   2.530000     Variance                 1.42360
                   Mode     0.151000     Range                    2.94800
                                         Interquartile Range      2.43700

              NOTE: The mode displayed is the smallest of 2 modes with a count of 2.


                                   Tests for Location: Mu0=0

                       Test           -Statistic-    -----p Value------

                       Student's t    t  7.861423    Pr > |t|    <.0001
                       Sign           M      13.5    Pr >= |M|   <.0001
                       Signed Rank    S       189    Pr >= |S|   <.0001


                                      Tests for Normality

                  Test                  --Statistic---    -----p Value------

                  Shapiro-Wilk          W     0.764426    Pr < W     <0.0001
                  Kolmogorov-Smirnov    D     0.249457    Pr > D     <0.0100
                  Cramer-von Mises      W-Sq  0.459846    Pr > W-Sq  <0.0050
                  Anderson-Darling      A-Sq   2.75771    Pr > A-Sq  <0.0050
```

```
                                     Quantiles (Definition 5)

                                      Quantile      Estimate

                                      100% Max         3.043
                                      99%              3.043
                                      95%              3.031
                                      90%              2.992

 Effect of temperature and moist on NO3 release    08:50 Sunday, November 28, 2008      10


                                     The UNIVARIATE Procedure
                                       Variable:  VI  (NO₃)

                                     Quantiles (Definition 5)

                                      Quantile      Estimate

                                      75% Q3           2.642
                                      50% Median       2.530
                                      25% Q1           0.205
                                      10%              0.151
                                      5%               0.151
                                      1%               0.095
                                      0% Min           0.095



                                       Extreme Observations

                                -----Lowest----        ----Highest----

                                 Value      Obs         Value      Obs

                                 0.095       19         2.912        7
                                 0.151       24         2.944       10
                                 0.151       22         2.992       11
                                 0.164       21         3.031        8
                                 0.191       23         3.043        9

                                         Frequency Counts

                  Percents                          Percents                          Percents
    Value Count  Cell   Cum         Value Count  Cell   Cum         Value Count  Cell   Cum

    0.095     1   3.7   3.7         1.964     1   3.7  40.7         2.638     1   3.7  74.1
    0.151     2   7.4  11.1         2.055     1   3.7  44.4         2.642     1   3.7  77.8
    0.164     1   3.7  14.8         2.410     1   3.7  48.1         2.864     1   3.7  81.5
    0.191     1   3.7  18.5         2.530     1   3.7  51.9         2.912     1   3.7  85.2
    0.193     1   3.7  22.2         2.554     1   3.7  55.6         2.944     1   3.7  88.9
    0.205     1   3.7  25.9         2.602     2   7.4  63.0         2.992     1   3.7  92.6
    0.273     1   3.7  29.6         2.609     1   3.7  66.7         3.031     1   3.7  96.3
    0.334     1   3.7  33.3         2.634     1   3.7  70.4         3.043     1   3.7 100.0
    1.956     1   3.7  37.0




 Effect of temperature and moist on NO3 release   08:50 Sunday, November 28, 2008         11
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```
              The UNIVARIATE Procedure
                Variable:  VI  (NO3)

  Stem Leaf                     #             Boxplot
     3 000                      3                |
     2 56666666999             11             +-----+
     2 0014                     4             |     |
     1                                        |  +  |
     1                                        |     |
     0                                        |     |
     0 122222233                9             +-----+
       ----+----+----+----+


                   Normal Probability Plot
3.25+                                    ++++ *   *
    |                         *** *****+** *
    |                       **  ++++
1.75+                     **++++
    |                   ++++
    |               ++++
0.25+     *   *++++** ****
     +----+----+----+----+----+----+----+----+----+----+
         -2        -1         0        +1        +2
```

A2.2 Unstable ‘Olifantsfontein’ sewage sludge amended soil NH_4^+ and NO_3^-

```
Effect of temperature and moist on NH4 and NO3 release   12:58 Sunday, December 12, 2008   1


                          Obs    Temp    Moist    Rep      V1        V2

                            1     T1      W1       1      5.842    0.265
                            2     T1      W1       2      6.201    0.186
                            3     T1      W1       3      6.041    0.265
                            4     T1      W2       1      5.773    0.040
                            5     T1      W2       2      5.813    0.159
                            6     T1      W2       3      5.773    0.159
                            7     T1      W3       1      5.494    0.345
                            8     T1      W3       2      5.391    0.225
                            9     T1      W3       3      5.431    0.265
                           10     T2      W1       1      4.183    1.300
                           11     T2      W1       2      5.111    1.180
                           12     T2      W1       3      4.274    1.220
                           13     T2      W2       1      5.364    0.383
                           14     T2      W2       2      5.404    0.483
                           15     T2      W2       3      5.404    0.443
                           16     T2      W3       1      6.626    0.848
                           17     T2      W3       2      6.466    0.728
                           18     T2      W3       3      6.546    0.768
                           19     T3      W1       1      7.014    0.223
                           20     T3      W1       2      7.452    0.143
                           21     T3      W1       3      7.293    0.203
                           22     T3      W2       1      8.711    0.172
                           23     T3      W2       2      9.069    0.133
                           24     T3      W2       3      9.308    0.172
                           25     T3      W3       1     10.490    0.146
                           26     T3      W3       2     10.730    0.106
                           27     T3      W3       3      9.852    0.106

Effect of temperature and moist on NH4 and NO3 release 12:58 Sunday, December 12, 2008     2

                                        The GLM Procedure
```

```
                                   Class Level Information

Class        Levels  Values

Temp              3  T1 T2 T3

Moist             3  W1 W2 W3

Rep               3  1 2 3

V1               25  4.183 4.274 5.111 5.364 5.391 5.404 5.431 5.494 5.773 5.813 5.842 6.041 6.201
                     6.466 6.546 6.626 7.014 7.293 7.452 8.711 9.069 9.308 9.852 10.49 10.73

V2               22  0.04 0.106 0.133 0.143 0.146 0.159 0.172 0.186 0.203 0.223 0.225 0.265 0.345
                     0.383 0.443 0.483 0.728 0.768 0.848 1.18 1.22 1.3

                                  Number of observations    27


Effect of temperature and moist on NH4 and NO3 release 12:58 Sunday, December 12, 2008      3

                                     The GLM Procedure

Dependent Variable: V1   NH4,

                                                 Sum of
       Source                      DF          Squares     Mean Square    F Value    Pr > F

       Model                        8      85.39071667     10.67383958     147.89    <.0001

       Error                       18       1.29909400      0.07217189

       Corrected Total             26      86.68981067


                        R-Square     Coeff Var      Root MSE       V1 Mean

                        0.985014      4.006221      0.268648      6.705778


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

       Temp                         2      64.12620156     32.06310078     444.26    <.0001
       Moist                        2      10.31011622      5.15505811      71.43    <.0001
       Temp*Moist                   4      10.95439889      2.73859972      37.95    <.0001


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

       Temp                         2      64.12620156     32.06310078     444.26    <.0001
       Moist                        2      10.31011622      5.15505811      71.43    <.0001
       Temp*Moist                   4      10.95439889      2.73859972      37.95    <.0001

Effect of temperature and moist on NH4 and NO3 release      12:58 Sunday, December 12, 2008    4

                                     The GLM Procedure

Dependent Variable: V2   NO3

                                                 Sum of
       Source                      DF          Squares     Mean Square    F Value    Pr > F

       Model                        8       3.42497363      0.42812170     165.31    <.0001

       Error                       18       0.04661533      0.00258974
```

```
       Corrected Total             26      3.47158896


                        R-Square     Coeff Var      Root MSE       V2 Mean

                        0.986572      12.88221      0.050889      0.395037


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

       Temp                         2       2.41788007      1.20894004     466.82    <.0001
       Moist                        2       0.44846052      0.22423026      86.58    <.0001
       Temp*Moist                   4       0.55863304      0.13965826      53.93    <.0001



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

       Temp                         2       2.41788007      1.20894004     466.82    <.0001
       Moist                        2       0.44846052      0.22423026      86.58    <.0001
       Temp*Moist                   4       0.55863304      0.13965826      53.93    <.0001





Effect of temperature and moist on NH4 and NO3 release 12:58 Sunday, December 12, 2008   5

                                     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                  18
                            Error Mean Square                   0.072172
                            Critical Value of Studentized Range  3.60930
                            Minimum Significant Difference        0.3232


                     Means with the same letter are not significantly different.


                      Tukey Grouping          Mean      N    Temp

                                   A        8.8799      9    T3

                                   B        5.7510      9    T1

                                   B        5.4864      9    T2
Effect of temperature and moist on NH4 and NO3 release 12:58 Sunday, December 12, 2008    6

                                     The GLM Procedure

                            Tukey's Studentized Range (HSD) Test for V2
```

```
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                  18
                           Error Mean Square                    0.00259
                           Critical Value of Studentized Range  3.60930
                           Minimum Significant Difference        0.0612


                    Means with the same letter are not significantly different.


                     Tukey Grouping          Mean      N    Temp

                                  A       0.81700      9    T2

                                  B       0.21211      9    T1

                                  B       0.15600      9    T3

Effect of temperature and moist on NH4 and NO3 release   12:58 Sunday, December 12, 2008    7


                                        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                  18
                           Error Mean Square                   0.072172
                           Critical Value of Studentized Range  3.60930
                           Minimum Significant Difference        0.3232


                   Means with the same letter are not significantly different.


                    Tukey Grouping          Mean      N    Moist

                                 A        7.4473      9    W3

                                 B        6.7354      9    W2

                                 C        5.9346      9    W1

Effect of temperature and moist on NH4 and NO3 release    12:58 Sunday, December 12, 2008   8


                                        The GLM Procedure

                           Tukey's Studentized Range (HSD) Test for V2

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                  18
                           Error Mean Square                    0.00259
                           Critical Value of Studentized Range  3.60930
                           Minimum Significant Difference        0.0612


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

```
                   Tukey Grouping          Mean      N    Moist

                                A       0.55389      9    W1

                                B       0.39300      9    W3

                                C       0.23822      9    W2

Effect of temperature and moist on NH4 and NO3 release   12:58 Sunday, December 12, 2008    9

                                       The GLM Procedure


 Level of     Level of            --------------V1-------------     --------------V2-------------
 Temp         Moist         N             Mean         Std Dev             Mean         Std Dev

 T1           W1            3        6.0280000      0.17985272       0.23866667      0.04561067
 T1           W2            3        5.7863333      0.02309401       0.11933333      0.06870468
 T1           W3            3        5.4386667      0.05192623       0.27833333      0.06110101
 T2           W1            3        4.5226667      0.51153918       1.23333333      0.06110101
 T2           W2            3        5.3906667      0.02309401       0.43633333      0.05033223
 T2           W3            3        6.5460000      0.08000000       0.78133333      0.06110101
 T3           W1            3        7.2530000      0.22172280       0.18966667      0.04163332
 T3           W2            3        9.0293333      0.30047019       0.15900000      0.02251666
 T3           W3            3       10.3573333      0.45378556       0.11933333      0.02309401


Effect of temperature and moist on NH4 and NO3 release   12:58 Sunday, December 12, 2008   10

                                       The GLM Procedure
                                      Least Squares Means

                                                 Standard                 LSMEAN
                   Temp        V1 LSMEAN            Error    Pr > |t|      Number

                   T1         5.75100000       0.08954942      <.0001           1
                   T2         5.48644444       0.08954942      <.0001           2
                   T3         8.87988889       0.08954942      <.0001           3


                              Least Squares Means for effect Temp
                              Pr > |t| for H0: LSMean(i)=LSMean(j)

                                     Dependent Variable: V1

                      i/j              1             2             3

                         1                      0.0512        <.0001
                         2        0.0512                      <.0001
                         3        <.0001        <.0001



                                                 Standard                 LSMEAN
                   Temp        V2 LSMEAN            Error    Pr > |t|      Number

                   T1         0.21211111       0.01696317      <.0001           1
                   T2         0.81700000       0.01696317      <.0001           2
                   T3         0.15600000       0.01696317      <.0001           3


                              Least Squares Means for effect Temp
                              Pr > |t| for H0: LSMean(i)=LSMean(j)
```

```
                                Dependent Variable: V2

                i/j              1              2              3

                   1                        <.0001         0.0311
                   2         <.0001                        <.0001
                   3         0.0311         <.0001


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


                                        Standard                     LSMEAN
                  Moist       V1 LSMEAN          Error    Pr > |t|      Number

                  W1         5.93455556      0.08954942      <.0001           1
                  W2         6.73544444      0.08954942      <.0001           2


Effect of temperature and moist on NH4 and NO3 release  12:58 Sunday, December 12, 2008   11

                                  The GLM Procedure
                                 Least Squares Means

                                        Standard                     LSMEAN
                  Moist       V1 LSMEAN          Error    Pr > |t|      Number

                  W3         7.44733333      0.08954942      <.0001           3


                             Least Squares Means for effect Moist
                             Pr > |t| for H0: LSMean(i)=LSMean(j)

                                 Dependent Variable: V1

                i/j              1              2              3

                   1                        <.0001         <.0001
                   2         <.0001                        <.0001
                   3         <.0001         <.0001



                                        Standard                     LSMEAN
                  Moist       V2 LSMEAN          Error    Pr > |t|      Number

                  W1         0.55388889      0.01696317      <.0001           1
                  W2         0.23822222      0.01696317      <.0001           2
                  W3         0.39300000      0.01696317      <.0001           3


                            Least Squares Means for effect Moist
                             Pr > |t| for H0: LSMean(i)=LSMean(j)

                                 Dependent Variable: V2

                i/j              1              2              3

                   1                        <.0001         <.0001
                   2         <.0001                        <.0001
                   3         <.0001         <.0001


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

```
                                              Standard                 LSMEAN
              Temp    Moist       V1 LSMEAN      Error    Pr > |t|     Number

              T1      W1          6.0280000  0.1551041      <.0001          1
              T1      W2          5.7863333  0.1551041      <.0001          2
              T1      W3          5.4386667  0.1551041      <.0001          3
              T2      W1          4.5226667  0.1551041      <.0001          4


Effect of temperature and moist on NH4 and NO3 release   12:58 Sunday, December 12, 2008   12

                                         The GLM Procedure
                                        Least Squares Means

                                              Standard                 LSMEAN
              Temp    Moist       V1 LSMEAN      Error    Pr > |t|     Number

              T2      W2          5.3906667  0.1551041      <.0001          5
              T2      W3          6.5460000  0.1551041      <.0001          6
              T3      W1          7.2530000  0.1551041      <.0001          7
              T3      W2          9.0293333  0.1551041      <.0001          8
              T3      W3         10.3573333  0.1551041      <.0001          9


                              Least Squares Means for effect Temp*Moist
                                 Pr > |t| for H0: LSMean(i)=LSMean(j)

                                       Dependent Variable: V1

  i/j          1          2          3          4          5          6          7          8          9

     1                0.2851     0.0151     <.0001     0.0094     0.0297     <.0001     <.0001     <.0001
     2     0.2851                0.1304     <.0001     0.0880     0.0028     <.0001     <.0001     <.0001
     3     0.0151     0.1304                0.0006     0.8292     <.0001     <.0001     <.0001     <.0001
     4     <.0001     <.0001     0.0006                0.0009     <.0001     <.0001     <.0001     <.0001
     5     0.0094     0.0880     0.8292     0.0009                <.0001     <.0001     <.0001     <.0001
     6     0.0297     0.0028     <.0001     <.0001     <.0001                0.0047     <.0001     <.0001
     7     <.0001     <.0001     <.0001     <.0001     <.0001     0.0047                <.0001     <.0001
     8     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001                <.0001
     9     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001



                                              Standard                 LSMEAN
              Temp    Moist       V2 LSMEAN      Error    Pr > |t|     Number

              T1      W1         0.23866667 0.02938106      <.0001          1
              T1      W2         0.11933333 0.02938106      0.0007          2
              T1      W3         0.27833333 0.02938106      <.0001          3
              T2      W1         1.23333333 0.02938106      <.0001          4
              T2      W2         0.43633333 0.02938106      <.0001          5
              T2      W3         0.78133333 0.02938106      <.0001          6
              T3      W1         0.18966667 0.02938106      <.0001          7
              T3      W2         0.15900000 0.02938106      <.0001          8
              T3      W3         0.11933333 0.02938106      0.0007          9



Effect of temperature and moist on NH4 and NO3 release 12:58 Sunday, December 12, 2008   13

                                         The GLM Procedure
                                        Least Squares Means

                              Least Squares Means for effect Temp*Moist
```

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

                                     Dependent Variable: V2

  i/j          1          2          3          4          5          6          7          8          9

     1                0.0101     0.3524     <.0001     0.0002     <.0001     0.2536     0.0712     0.0101
     2     0.0101                0.0012     <.0001     <.0001     <.0001     0.1078     0.3524     1.0000
     3     0.3524     0.0012                <.0001     0.0013     <.0001     0.0469     0.0101     0.0012
     4     <.0001     <.0001     <.0001                <.0001     <.0001     <.0001     <.0001     <.0001
     5     0.0002     <.0001     0.0013     <.0001                <.0001     <.0001     <.0001     <.0001
     6     <.0001     <.0001     <.0001     <.0001     <.0001                <.0001     <.0001     <.0001
     7     0.2536     0.1078     0.0469     <.0001     <.0001     <.0001                0.4700     0.1078
     8     0.0712     0.3524     0.0101     <.0001     <.0001     <.0001     0.4700                0.3524
     9     0.0101     1.0000     0.0012     <.0001     <.0001     <.0001     0.1078     0.3524

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


Effect of temperature and moist on NH4 and NO3 release   12:58 Sunday, December 12, 2008   14


                                    The UNIVARIATE Procedure
                                     Variable:  V1  (NH4,)

                                            Moments

                 N                          27    Sum Weights                 27
                 Mean               6.70577778    Sum Observations       181.056
                 Std Deviation      1.82598562    Variance            3.33422349
                 Skewness            0.9495203    Kurtosis            -0.0641734
                 Uncorrected SS     1300.81111    Corrected SS        86.6898107
                 Coeff Variation    27.2300348    Std Error Mean       0.3514111


                                   Basic Statistical Measures

                         Location                    Variability

                      Mean     6.705778     Std Deviation            1.82599
                      Median   6.041000     Variance                 3.33422
                      Mode     5.404000     Range                    6.54700
                                            Interquartile Range      2.04800

              NOTE: The mode displayed is the smallest of 2 modes with a count of 2.


                                   Tests for Location: Mu0=0

                        Test           -Statistic-    -----p Value------

                        Student's t    t  19.08243    Pr > |t|    <.0001
                        Sign           M      13.5    Pr >= |M|   <.0001
                        Signed Rank    S       189    Pr >= |S|   <.0001




                                      Tests for Normality

                   Test                  --Statistic---    -----p Value------

                   Shapiro-Wilk          W     0.883629    Pr < W      0.0058
                   Kolmogorov-Smirnov    D     0.184091    Pr > D      0.0194
                   Cramer-von Mises      W-Sq  0.238222    Pr > W-Sq  <0.0050
                   Anderson-Darling      A-Sq  1.310152    Pr > A-Sq  <0.0050
```

```
                                   Quantiles (Definition 5)

                                    Quantile      Estimate

                                    100% Max        10.730
                                    99%             10.730
                                    95%             10.490
                                    90%              9.852
```

```
Effect of temperature and moist on NH4 and NO3 release  12:58 Sunday, December 12, 2008    15

                                   The UNIVARIATE Procedure
                                    Variable:  V1  (NH4,)

                                   Quantiles (Definition 5)

                                    Quantile      Estimate

                                    75% Q3           7.452
                                    50% Median       6.041
                                    25% Q1           5.404
                                    10%              5.111
                                    5%               4.274
                                    1%               4.183
                                    0% Min           4.183


                                     Extreme Observations

                               -----Lowest----        -----Highest----

                                Value      Obs           Value      Obs

                                4.183       10           9.069       23
                                4.274       12           9.308       24
                                5.111       11           9.852       27
                                5.364       13          10.490       25
                                5.391        8          10.730       26


                                      Frequency Counts

                  Percents                          Percents                          Percents
    Value Count  Cell   Cum          Value Count  Cell   Cum          Value Count  Cell   Cum

    4.183     1   3.7   3.7          5.813     1   3.7  44.4          7.293     1   3.7  74.1
    4.274     1   3.7   7.4          5.842     1   3.7  48.1          7.452     1   3.7  77.8
    5.111     1   3.7  11.1          6.041     1   3.7  51.9          8.711     1   3.7  81.5
    5.364     1   3.7  14.8          6.201     1   3.7  55.6          9.069     1   3.7  85.2
    5.391     1   3.7  18.5          6.466     1   3.7  59.3          9.308     1   3.7  88.9
    5.404     2   7.4  25.9          6.546     1   3.7  63.0          9.852     1   3.7  92.6
    5.431     1   3.7  29.6          6.626     1   3.7  66.7         10.490     1   3.7  96.3
    5.494     1   3.7  33.3          7.014     1   3.7  70.4         10.730     1   3.7 100.0
    5.773     2   7.4  40.7
```

```
Effect of temperature and moist on NH4 and NO3 release  12:58 Sunday, December 12, 2008    16

                                     The UNIVARIATE Procedure
                                      Variable:  V1  (NH4,)

                         Stem Leaf                     #             Boxplot
                           10 57                       2                0
                            9 139                      3                |
                            8 7                        1                |
                            7 035                      3             +-----+
                            6 02556                    5             *--+--*
                            5 14444458888             11             +-----+
                            4 23                       2                |
                              ----+----+----+----+


                                          Normal Probability Plot
                       10.5+                                         *  +*+++
                           |                                   ** *+++++
                           |                                 *++++
                        7.5+                           +++***
                           |                      +++*****
                           |                * **+*+*** **
                        4.5+     *   * +++++
                            +----+----+----+----+----+----+----+----+----+----+
                                -2        -1         0        +1        +2
```

```
Effect of temperature and moist on NH4 and NO3 release  12:58 Sunday, December 12, 2008   17

                                     The UNIVARIATE Procedure
                                       Variable:  V2  (NO3)

                                             Moments

                 N                          27    Sum Weights                 27
                 Mean               0.39503704    Sum Observations        10.666
                 Std Deviation      0.36540752    Variance            0.13352265
                 Skewness           1.48548872    Kurtosis            1.09630973
                 Uncorrected SS       7.685054    Corrected SS        3.47158896
                 Coeff Variation    92.4995586    Std Error Mean      0.07032271


                                    Basic Statistical Measures

                          Location                    Variability

                      Mean     0.395037     Std Deviation            0.36541
                      Median   0.225000     Variance                 0.13352
                      Mode     0.265000     Range                    1.26000
                                            Interquartile Range      0.32400


                                    Tests for Location: Mu0=0

                         Test           -Statistic-    -----p Value------

                         Student's t    t  5.617489    Pr > |t|    <.0001
                         Sign           M      13.5    Pr >= |M|   <.0001
                         Signed Rank    S       189    Pr >= |S|   <.0001
```

```
                                       Tests for Normality

                    Test                  --Statistic---    -----p Value------

                    Shapiro-Wilk          W     0.770147    Pr < W    <0.0001
                    Kolmogorov-Smirnov    D      0.26866    Pr > D    <0.0100
                    Cramer-von Mises      W-Sq  0.457782    Pr > W-Sq <0.0050
                    Anderson-Darling      A-Sq  2.519833    Pr > A-Sq <0.0050


                                     Quantiles (Definition 5)

                                      Quantile      Estimate

                                      100% Max         1.300
                                      99%              1.300
                                      95%              1.220
                                      90%              1.180
                                      75% Q3           0.483
                                      50% Median       0.225


Effect of temperature and moist on NH4 and NO3 release   12:58 Sunday, December 12, 2008   18

                                     The UNIVARIATE Procedure
                                       Variable:  V2  (NO3)

                                     Quantiles (Definition 5)

                                      Quantile      Estimate

                                      25% Q1           0.159
                                      10%              0.106
                                      5%               0.106
                                      1%               0.040
                                      0% Min           0.040


                                       Extreme Observations

                              -----Lowest----       ----Highest----

                               Value      Obs        Value      Obs

                               0.040        4        0.768       18
                               0.106       27        0.848       16
                               0.106       26        1.180       11
                               0.133       23        1.220       12
                               0.143       20        1.300       10


                                         Frequency Counts

                  Percents                          Percents                         Percents
    Value Count  Cell   Cum           Value Count  Cell  Cum          Value Count  Cell   Cum

    0.040     1   3.7   3.7           0.203     1   3.7  44.4         0.483     1   3.7  77.8
    0.106     2   7.4  11.1           0.223     1   3.7  48.1         0.728     1   3.7  81.5
    0.133     1   3.7  14.8           0.225     1   3.7  51.9         0.768     1   3.7  85.2
    0.143     1   3.7  18.5           0.265     3  11.1  63.0         0.848     1   3.7  88.9
    0.146     1   3.7  22.2           0.345     1   3.7  66.7         1.180     1   3.7  92.6
    0.159     2   7.4  29.6           0.383     1   3.7  70.4         1.220     1   3.7  96.3
    0.172     2   7.4  37.0           0.443     1   3.7  74.1         1.300     1   3.7 100.0
    0.186     1   3.7  40.7
```

```
Effect of temperature and moist on NH4 and NO3 release   12:58 Sunday, December 12, 2008    19

                                     The UNIVARIATE Procedure
                                       Variable:  V2  (NO3)

                         Stem Leaf                      #            Boxplot
                           12 20                        2               0
                           10 8                         1               0
                            8 5                         1               |
                            6 37                        2               |
                            4 48                        2            +--+--+
                            2 02266648                  8            *-----*
                            0 41134566779              11            +-----+
                              ----+----+----+----+
                          Multiply Stem.Leaf by 10**-1


                                          Normal Probability Plot
                        1.3+                                         *   *  +++
                           |                                      *   ++++++
                           |                                    *+++++
                        0.7+                               ++*+*+
                           |                          +++++**
                           |                    +++**** ***
                        0.1+     *   *    **+**+* *
                            +----+----+----+----+----+----+----+----+----+----+
                                -2        -1         0        +1        +2
```

A$_{2.3}$ Unstable Sasol sludge amended soil NH_4^+ and NO_3^-

```
N forms in unstable Sasol sludge amended soils      58 Monday, December 13, 2008      1


                          Obs    Temp    Moist    rep      V1       V2

                            1     T1      W1       1     6.147     0.586
```

```
                        2      T1      W1      2      6.107     0.785
                        3      T1      W1      3      6.186     0.705
                        4      T1      W2      1      5.719     0.666
                        5      T1      W2      2      5.958     0.634
                        6      T1      W2      3      5.942     0.658
                        7      T1      W3      1      4.181     0.657
                        8      T1      W3      2      4.381     0.721
                        9      T1      W3      3      4.269     0.705
                       10      T2      W1      1      0.000    10.217
                       11      T2      W1      2      0.000     9.141
                       12      T2      W1      3      0.000     9.898
                       13      T2      W2      1      0.000     7.169
                       14      T2      W2      2      0.000     7.607
                       15      T2      W2      3      0.000     7.647
                       16      T2      W3      1      0.174     7.056
                       17      T2      W3      2      0.214     7.016
                       18      T2      W3      3      0.230     7.454
                       19      T3      W1      1      5.313     0.178
                       20      T3      W1      2      5.465     0.226
                       21      T3      W1      3      5.544     0.210
                       22      T3      W2      1      6.050     0.168
                       23      T3      W2      2      5.970     0.208
                       24      T3      W2      3      5.890     0.188
                       25      T3      W3      1      7.230     0.111
                       26      T3      W3      2      7.390     0.095
                       27      T3      W3      3      7.230     0.079


N forms in unstable Sasol sludge       21:58 Monday, December 13, 2008        2


                                        The GLM Procedure

                                     Class Level Information

Class        Levels  Values

Temp              3  T1 T2 T3

Moist             3  W1 W2 W3

rep               3  1 2 3

V1               21  0 0.174 0.214 0.23 4.181 4.269 4.381 5.313 5.465 5.544 5.719 5.89 5.942 5.958
                     5.97 6.05 6.107 6.147 6.186 7.23 7.39

V2               26  0.079 0.095 0.111 0.168 0.178 0.188 0.208 0.21 0.226 0.586 0.634 0.657 0.658
                     0.666 0.705 0.721 0.785 7.016 7.056 7.169 7.454 7.607 7.647 9.141 9.898 10.217
                                         Number of observations    27
N forms in unstable Sasol sludge               21:58 Monday, December 13, 2008     3


                                        The GLM Procedure

Dependent Variable: V1   NH4,

                                                Sum of
       Source                      DF          Squares     Mean Square    F Value    Pr > F

       Model                       10      213.7838917      21.3783892    3737.42    <.0001

       Error                       16        0.0915215       0.0057201

       Corrected Total             26      213.8754132


                       R-Square     Coeff Var      Root MSE       V1 Mean

                       0.999572      1.933938      0.075631      3.910741
```

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

       Temp                         2     202.1543016     101.0771508    17670.5    <.0001
       Moist                        2       0.0344281       0.0172140       3.01    0.0777
       Temp*Moist                   4      11.5686655       2.8921664     505.62    <.0001
       rep                          2       0.0264965       0.0132483       2.32    0.1308


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

       Temp                         2     202.1543016     101.0771508    17670.5    <.0001
       Moist                        2       0.0344281       0.0172140       3.01    0.0777
       Temp*Moist                   4      11.5686655       2.8921664     505.62    <.0001
       rep                          2       0.0264965       0.0132483       2.32    0.1308
```

```
N forms in unstable Sasol sludge       21:58 Monday, December 13, 2008           4

                                      The GLM Procedure

Dependent Variable: V2   NO3

                                              Sum of
       Source                      DF         Squares     Mean Square    F Value    Pr > F

       Model                       10     370.1365704      37.0136570     719.47    <.0001

       Error                       16       0.8231346       0.0514459

       Corrected Total             26     370.9597050

                        R-Square     Coeff Var      Root MSE       V2 Mean

                        0.997781      7.580685      0.226817      2.992037

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

       Temp                         2     358.1249639     179.0624819    3480.60    <.0001
       Moist                        2       4.2575299       2.1287649      41.38    <.0001
       Temp*Moist                   4       7.6830899       1.9207725      37.34    <.0001
       rep                          2       0.0709867       0.0354934       0.69    0.5159
```

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

        Temp                         2     358.1249639     179.0624819    3480.60    <.0001
        Moist                        2       4.2575299       2.1287649      41.38    <.0001
        Temp*Moist                   4       7.6830899       1.9207725      37.34    <.0001
        rep                          2       0.0709867       0.0354934       0.69    0.5159




N forms in unstable Sasol sludge               21:58 Monday, December 13, 2008     5


                                    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                  16
                           Error Mean Square                    0.00572
                           Critical Value of Studentized Range  3.64914
                           Minimum Significant Difference         0.092




Means with the same letter are not significantly different.


                     Tukey Grouping          Mean      N    Temp

                                  A       6.23133      9    T3

                                  B       5.43222      9    T1

                                  C       0.06867      9    T2




N forms in unstable Sasol sludge            21:58 Monday, December 13, 2008       6


                                    The GLM Procedure

                           Tukey's Studentized Range (HSD) Test for V2

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                  16
                           Error Mean Square                   0.051446
                           Critical Value of Studentized Range  3.64914
                           Minimum Significant Difference        0.2759


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

```
                    Tukey Grouping          Mean      N    Temp

                                 A        8.1339      9    T2

                                 B        0.6797      9    T1

                                 C        0.1626      9    T3

N forms in unstable Sasol sludge        21:58 Monday, December 13, 2008        7

                                   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                     16
                          Error Mean Square                       0.00572
                          Critical Value of Studentized Range   3.64914
                          Minimum Significant Difference          0.092


                   Means with the same letter are not significantly different.

                   Tukey Grouping          Mean      N    Moist

                                A       3.94767      9    W2

                                A       3.92211      9    W3

                                A       3.86244      9    W1


N forms in unstable Sasol sludge    21:58 Monday, December 13, 2008               8

                                   The GLM Procedure

                          Tukey's Studentized Range (HSD) Test for V2

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                     16
                          Error Mean Square                      0.051446
                          Critical Value of Studentized Range   3.64914
                          Minimum Significant Difference         0.2759


                  Means with the same letter are not significantly different.


                   Tukey Grouping          Mean      N    Moist

                                A        3.5496      9    W1

                                B        2.7717      9    W2

                                B        2.6549      9    W3
```

```
N forms in unstable Sasol sludge     21:58 Monday, December 13, 2008       9

                                 The GLM Procedure

 Level of     Level of            --------------V1-------------     --------------V2-------------
 Temp         Moist        N             Mean          Std Dev             Mean          Std Dev

 T1           W1           3       6.14666667       0.03950105       0.69200000       0.10013491
 T1           W2           3       5.87300000       0.13360763       0.65266667       0.01665333
 T1           W3           3       4.27700000       0.10023971       0.69433333       0.03330666
 T2           W1           3       0.00000000       0.00000000       9.75200000       0.55265812
 T2           W2           3       0.00000000       0.00000000       7.47433333       0.26518170
 T2           W3           3       0.20600000       0.02884441       7.17533333       0.24215973
 T3           W1           3       5.44066667       0.11740670       0.20466667       0.02444040
 T3           W2           3       5.97000000       0.08000000       0.18800000       0.02000000
 T3           W3           3       7.28333333       0.09237604       0.09500000       0.01600000


N forms in unstable Sasol sludge             21:58 Monday, December 13, 2008     10

                                 The GLM Procedure
                                Least Squares Means

                                          Standard                    LSMEAN
                   Temp       V1 LSMEAN       Error    Pr > |t|      Number

                   T1        5.43222222  0.02521043      <.0001           1
                   T2        0.06866667  0.02521043      0.0150           2
                   T3        6.23133333  0.02521043      <.0001           3


                           Least Squares Means for effect Temp
                           Pr > |t| for H0: LSMean(i)=LSMean(j)

                                Dependent Variable: V1

                      i/j              1              2              3

                         1                       <.0001         <.0001
                         2        <.0001                        <.0001
                         3        <.0001         <.0001


                                          Standard                    LSMEAN
                   Temp       V2 LSMEAN       Error    Pr > |t|      Number

                   T1        0.67966667  0.07560564      <.0001           1
                   T2        8.13388889  0.07560564      <.0001           2
                   T3        0.16255556  0.07560564      0.0472           3


                           Least Squares Means for effect Temp
                           Pr > |t| for H0: LSMean(i)=LSMean(j)

                                Dependent Variable: V2

                      i/j              1              2              3

                         1                       <.0001         0.0002
                         2        <.0001                        <.0001
                         3        0.0002         <.0001
```

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


                                     Standard                   LSMEAN
                  Moist       V1 LSMEAN         Error    Pr > |t|      Number

                  W1         3.86244444    0.02521043      <.0001           1
                  W2         3.94766667    0.02521043      <.0001           2



N forms in unstable Sasol sewage sludge            21:58 Monday, December 13, 2008       11

                                  The GLM Procedure
                                 Least Squares Means

                                     Standard                   LSMEAN
                  Moist       V1 LSMEAN         Error    Pr > |t|      Number

                  W3         3.92211111    0.02521043      <.0001           3



                                Least Squares Means for effect Moist
                                Pr > |t| for H0: LSMean(i)=LSMean(j)

                                       Dependent Variable: V1

                          i/j              1             2             3

                             1                      0.0295        0.1137
                             2        0.0295                      0.4838
                             3        0.1137        0.4838



                                     Standard                   LSMEAN
                  Moist       V2 LSMEAN         Error    Pr > |t|      Number

                  W1         3.54955556    0.07560564      <.0001           1
                  W2         2.77166667    0.07560564      <.0001           2
                  W3         2.65488889    0.07560564      <.0001           3


                                Least Squares Means for effect Moist
                                Pr > |t| for H0: LSMean(i)=LSMean(j)

                                       Dependent Variable: V2

                          i/j              1             2             3

                             1                      <.0001        <.0001
                             2        <.0001                      0.2909
                             3        <.0001        0.2909



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

```
                             Standard           LSMEAN
              Temp    Moist         V1 LSMEAN           Error     Pr > |t|        Number

              T1      W1         6.14666667      0.04366575       <.0001             1
              T1      W2         5.87300000      0.04366575       <.0001             2
              T1      W3         4.27700000      0.04366575       <.0001             3
              T2      W1        -0.00000000      0.04366575       1.0000             4



N forms in unstable Sasol sludge              21:58 Monday, December 13, 2008    12


                                     The GLM Procedure
                                    Least Squares Means

                                                     Standard                 LSMEAN
              Temp    Moist          V1 LSMEAN          Error     Pr > |t|        Number

              T2      W2        -0.00000000      0.04366575       1.0000             5
              T2      W3         0.20600000      0.04366575       0.0002             6
              T3      W1         5.44066667      0.04366575       <.0001             7
              T3      W2         5.97000000      0.04366575       <.0001             8
              T3      W3         7.28333333      0.04366575       <.0001             9



                         Least Squares Means for effect Temp*Moist
                            Pr > |t| for H0: LSMean(i)=LSMean(j)

                                  Dependent Variable: V1

  i/j           1          2          3          4          5          6          7          8          9

     1                 0.0004     <.0001     <.0001     <.0001     <.0001     <.0001     0.0113     <.0001
     2     0.0004                 <.0001     <.0001     <.0001     <.0001     <.0001     0.1358     <.0001
     3     <.0001     <.0001                 <.0001     <.0001     <.0001     <.0001     <.0001     <.0001
     4     <.0001     <.0001     <.0001                 1.0000     0.0042     <.0001     <.0001     <.0001
     5     <.0001     <.0001     <.0001     1.0000                 0.0042     <.0001     <.0001     <.0001
     6     <.0001     <.0001     <.0001     0.0042     0.0042                <.0001     <.0001     <.0001
     7     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001                <.0001     <.0001
     8     0.0113     0.1358     <.0001     <.0001     <.0001     <.0001     <.0001                <.0001
     9     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001


                                                     Standard                 LSMEAN
              Temp    Moist          V2 LSMEAN          Error     Pr > |t|        Number

              T1      W1         0.69200000      0.13095281       <.0001             1
              T1      W2         0.65266667      0.13095281       0.0001             2
              T1      W3         0.69433333      0.13095281       <.0001             3
              T2      W1         9.75200000      0.13095281       <.0001             4
              T2      W2         7.47433333      0.13095281       <.0001             5
              T2      W3         7.17533333      0.13095281       <.0001             6
              T3      W1         0.20466667      0.13095281       0.1376             7
              T3      W2         0.18800000      0.13095281       0.1704             8
              T3      W3         0.09500000      0.13095281       0.4787             9

N forms in unstable Sasol sludge              21:58 Monday, December 13, 2008     13


                                     The GLM Procedure
                                    Least Squares Means

                         Least Squares Means for effect Temp*Moist
                            Pr > |t| for H0: LSMean(i)=LSMean(j)

                                  Dependent Variable: V2
```

```
  i/j          1          2          3          4          5          6          7          8          9

     1                0.8345     0.9901     <.0001     <.0001     <.0001     0.0181     0.0151     0.0053
     2    0.8345                 0.8248     <.0001     <.0001     <.0001     0.0278     0.0232     0.0083
     3    0.9901      0.8248                <.0001     <.0001     <.0001     0.0177     0.0147     0.0052
     4    <.0001      <.0001     <.0001                <.0001     <.0001     <.0001     <.0001     <.0001
     5    <.0001      <.0001     <.0001     <.0001                0.1260     <.0001     <.0001     <.0001
     6    <.0001      <.0001     <.0001     <.0001     0.1260                <.0001     <.0001     <.0001
     7    0.0181      0.0278     0.0177     <.0001     <.0001     <.0001                0.9294     0.5620
     8    0.0151      0.0232     0.0147     <.0001     <.0001     <.0001     0.9294                0.6224
     9    0.0053      0.0083     0.0052     <.0001     <.0001     <.0001     0.5620     0.6224


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


N forms in unstable Sasol sludge      21:58 Monday, December 13, 2008         14


                                      The UNIVARIATE Procedure
                                       Variable:  V1  (NH4,)

                                              Moments

                 N                          27    Sum Weights                 27
                 Mean               3.91074074    Sum Observations        105.59
                 Std Deviation      2.86809648    Variance            8.22597743
                 Skewness           -0.5324572    Kurtosis            -1.5540873
                 Uncorrected SS     626.810528    Corrected SS        213.875413
                 Coeff Variation    73.3389573    Std Error Mean      0.55196543


                                     Basic Statistical Measures

                          Location                    Variability

                      Mean     3.910741     Std Deviation            2.86810
                      Median   5.465000     Variance                 8.22598
                      Mode     0.000000     Range                    7.39000
                                            Interquartile Range      5.87600
```

```
                                        Tests for Location: Mu0=0

                         Test           -Statistic-    -----p Value------

                         Student's t    t  7.085119    Pr > |t|    <.0001
                         Sign           M      10.5    Pr >= |M|   <.0001
                         Signed Rank    S     115.5    Pr >= |S|   <.0001




                                       Tests for Normality

                    Test                  --Statistic---    -----p Value------

                    Shapiro-Wilk          W     0.781574    Pr < W     <0.0001
                    Kolmogorov-Smirnov    D     0.243105    Pr > D     <0.0100
                    Cramer-von Mises      W-Sq  0.426203    Pr > W-Sq  <0.0050
                    Anderson-Darling      A-Sq  2.582121    Pr > A-Sq  <0.0050

                                     Quantiles (Definition 5)

                                      Quantile      Estimate

                                      100% Max         7.390
                                      99%              7.390
                                      95%              7.230
                                      90%              7.230
                                      75% Q3           6.050
                                      50% Median       5.465



N forms in unstable Sasol sewage sludge           21:58 Monday, December 13, 2008     15

                                     The UNIVARIATE Procedure
                                      Variable:  V1  (NH4,)

                                     Quantiles (Definition 5)

                                      Quantile      Estimate

                                      25% Q1           0.174
                                      10%              0.000
                                      5%               0.000
                                      1%               0.000
                                      0% Min           0.000


                                       Extreme Observations

                              ----Lowest----        ----Highest----

                              Value      Obs         Value      Obs

                                  0       15         6.147        1
                                  0       14         6.186        3
                                  0       13         7.230       25
                                  0       12         7.230       27
                                  0       11         7.390       26

                                         Frequency Counts

                  Percents                          Percents                          Percents
```

```
    Value Count  Cell   Cum           Value Count  Cell   Cum           Value Count  Cell   Cum

    0.000     6  22.2  22.2           5.313     1   3.7  48.1           5.970     1   3.7  74.1
    0.174     1   3.7  25.9           5.465     1   3.7  51.9           6.050     1   3.7  77.8
    0.214     1   3.7  29.6           5.544     1   3.7  55.6           6.107     1   3.7  81.5
    0.230     1   3.7  33.3           5.719     1   3.7  59.3           6.147     1   3.7  85.2
    4.181     1   3.7  37.0           5.890     1   3.7  63.0           6.186     1   3.7  88.9
    4.269     1   3.7  40.7           5.942     1   3.7  66.7           7.230     2   7.4  96.3
    4.381     1   3.7  44.4           5.958     1   3.7  70.4           7.390     1   3.7 100.0
```

```
N forms in unstable Sasol sludge                  21:58 Monday, December 13, 2008       16

                                     The UNIVARIATE Procedure
                                      Variable:  V1  (NH4,)

                         Stem Leaf                     #             Boxplot
                            7 224                      3                |
                            6 000112                   6             +-----+
                            5 355799                   6             *-----*
                            4 234                      3             |     |
                            3                                        |  +  |
                            2                                        |     |
                            1                                        |     |
                            0 000000222                9             +-----+
                              ----+----+----+----+


                                          Normal Probability Plot
                        7.5+                                    ++*+ *   *
                           |                                **+**
                           |                        ********+
                           |                     ***  +++
                           |                      ++++
                           |                   +++
                           |               ++++
                        0.5+     *   *  *+** ****
                            +----+----+----+----+----+----+----+----+----+----+
                                -2        -1         0        +1        +2
```

```
N forms in unstable Sasol sludge            21:58 Monday, December 13, 2008   17


                                     The UNIVARIATE Procedure
                                       Variable:  V2 (NO3)

                                             Moments

                 N                          27    Sum Weights                 27
                 Mean               2.99203704    Sum Observations        80.785
                 Std Deviation      3.77725839    Variance             14.267681
                 Skewness           0.86063538    Kurtosis            -1.1365031
                 Uncorrected SS     612.671417    Corrected SS        370.959705
                 Coeff Variation    126.243704    Std Error Mean      0.72693372


                                    Basic Statistical Measures

                          Location                    Variability

                      Mean     2.992037     Std Deviation            3.77726
                      Median   0.666000     Variance                14.26768
                      Mode     0.705000     Range                   10.13800
```

```
                                            Interquartile Range      6.96100


                                    Tests for Location: Mu0=0

                         Test           -Statistic-    -----p Value------

                         Student's t    t   4.11597    Pr > |t|    0.0003
                         Sign           M      13.5    Pr >= |M|   <.0001
                         Signed Rank    S       189    Pr >= |S|   <.0001




                                       Tests for Normality

                    Test                  --Statistic---    -----p Value------

                    Shapiro-Wilk          W     0.705827    Pr < W     <0.0001
                    Kolmogorov-Smirnov    D     0.387156    Pr > D     <0.0100
                    Cramer-von Mises      W-Sq  0.733721    Pr > W-Sq  <0.0050
                    Anderson-Darling      A-Sq  3.822379    Pr > A-Sq  <0.0050
                                     Quantiles (Definition 5)

                                      Quantile      Estimate

                                      100% Max        10.217
                                      99%             10.217
                                      95%              9.898
                                      90%              9.141
                                      75% Q3           7.169
                                      50% Median       0.666
N forms in unstable Sasol sewage sludge           21:58 Monday, December 13, 2008   18


                                     The UNIVARIATE Procedure
                                       Variable:  V2  (NO3)

                                     Quantiles (Definition 5)

                                      Quantile      Estimate

                                      25% Q1           0.208
                                      10%              0.111
                                      5%               0.095
                                      1%               0.079
                                      0% Min           0.079


                                       Extreme Observations

                             -----Lowest----        -----Highest----

                              Value      Obs          Value      Obs

                              0.079       27          7.607       14
                              0.095       26          7.647       15
                              0.111       25          9.141       11
                              0.168       22          9.898       12
                              0.178       19         10.217       10


                                         Frequency Counts

                  Percents                          Percents                          Percents
    Value Count  Cell   Cum           Value Count  Cell   Cum           Value Count  Cell   Cum

    0.079     1   3.7   3.7           0.586     1   3.7  37.0           7.056     1   3.7  74.1
```

```
    0.095     1   3.7   7.4          0.634     1   3.7  40.7          7.169     1   3.7  77.8
    0.111     1   3.7  11.1          0.657     1   3.7  44.4          7.454     1   3.7  81.5
    0.168     1   3.7  14.8          0.658     1   3.7  48.1          7.607     1   3.7  85.2
    0.178     1   3.7  18.5          0.666     1   3.7  51.9          7.647     1   3.7  88.9
    0.188     1   3.7  22.2          0.705     2   7.4  59.3          9.141     1   3.7  92.6
    0.208     1   3.7  25.9          0.721     1   3.7  63.0          9.898     1   3.7  96.3
    0.210     1   3.7  29.6          0.785     1   3.7  66.7         10.217     1   3.7 100.0
    0.226     1   3.7  33.3          7.016     1   3.7  70.4



N forms in unstable Sasol sewage sludge           21:58 Monday, December 13, 2008      19

                                     The UNIVARIATE Procedure
                                       Variable:  V2  (NO3)

                         Stem Leaf                     #             Boxplot
                           10 2                        1                |
                            9 19                       2                |
                            8                                           |
                            7 012566                   6             +-----+
                            6                                        |     |
                            5                                        |     |
                            4                                        |     |
                            3                                        |  +  |
                            2                                        |     |
                            1                                        |     |
                            0 111222222667777778      18             *-----*
                              ----+----+----+----+



                                         Normal Probability Plot
                       10.5+                                           +*+
                           |                                     *  *++
                           |                                      ++
                           |                             **** **++
                           |                                +++
                        5.5+                              ++
                           |                           +++
                           |                         ++
                           |                      +++
                           |                   +++
                        0.5+     *   *  * ** ********** **
                            +----+----+----+----+----+----+----+----+----+----+
                                -2        -1         0        +1        +2
```

A_3. Statistical analysis for incubation time and water potential effect on net N release

```
Effect of incubation time on net N release for stable ‘Vlakplaas’- at 45 ° C            1

                                                           19:24 Thursday, November 25, 2008

                              Obs    Days    Mois    Rep      VI

                                1      1      W1      1     3.332
                                2      1      W1      2     3.173
                                3      1      W1      3     3.252
                                4      1      W2      1     3.002
                                5      1      W2      2     2.994
                                6      1      W2      3     2.998
                                7      1      W3      1     2.785
                                8      1      W3      2     2.753
                                9      1      W3      3     2.745
                               10      7      W1      1     3.141
                               11      7      W1      2     3.102
                               12      7      W1      3     3.087
                               13      7      W2      1     4.264
                               14      7      W2      2     3.390
                               15      7      W2      3     3.564
                               16      7      W3      1     3.411
                               17      7      W3      2     2.497
                               18      7      W3      3     2.791
                               19     14      W1      1     3.872
                               20     14      W1      2     3.991
                               21     14      W1      3     3.846
                               22     14      W2      1     3.784
                               23     14      W2      2     3.844
                               24     14      W2      3     3.825
                               25     14      W3      1     3.947
                               26     14      W3      2     3.804
                               27     14      W3      3     3.848
                               28     28      W1      1     2.898
                               29     28      W1      2     2.854
                               30     28      W1      3     2.963
                               31     28      W2      1     3.736
                               32     28      W2      2     3.780
                               33     28      W2      3     3.748
                               34     28      W3      1     3.448
                               35     28      W3      2     2.952
                               36     28      W3      3     3.259
                               37     56      W1      1     3.115
                               38     56      W1      2     2.996
                               39     56      W1      3     3.055
                               40     56      W2      1     2.958
                               41     56      W2      2     3.526
                               42     56      W2      3     2.906
                               43     56      W3      1     3.324
                               44     56      W3      2     3.249
                               45     56      W3      3     3.259




Effect of incubation time on net N release for stable ‘Vlakplaas’- at 45 ° C          2
                                                     19:24 Thursday, November 25, 2008

                                       The GLM Procedure

                                    Class Level Information

Class        Levels  Values

Days              5  1 14 28 56 7
```

```
Mois             3  W1 W2 W3

Rep              3  1 2 3

VI              44  2.497 2.745 2.753 2.785 2.791 2.854 2.898 2.906 2.952 2.958 2.963 2.994 2.996
                    2.998 3.002 3.055 3.087 3.102 3.115 3.141 3.173 3.249 3.252 3.259 3.324 3.332
                    3.39 3.411 3.448 3.526 3.564 3.736 3.748 3.78 3.784 3.804 3.825 3.844 3.846
                    3.848 3.872 3.947 3.991 4.264

                                   Number of observations    45


Effect of incubation time on net N release for stable ‘Vlakplaas’- at 45 ° C                3
                                                        19:24 Thursday, November 25, 2008

                                        The GLM Procedure

Dependent Variable: VI   N03

                                              Sum of
       Source                      DF        Squares     Mean Square    F Value    Pr > F

       Model                       14     6.54535458      0.46752533      10.94    <.0001

       Error                       30     1.28249800      0.04274993

       Corrected Total             44     7.82785258


                       R-Square     Coeff Var      Root MSE       VI Mean

                       0.836162      6.241598      0.206761      3.312622


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

       Days                         4     3.84301658      0.96075414      22.47    <.0001
       Mois                         2     0.70371551      0.35185776       8.23    0.0014
       Days*Mois                    8     1.99862249      0.24982781       5.84    0.0002


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

       Days                         4     3.84301658      0.96075414      22.47    <.0001
       Mois                         2     0.70371551      0.35185776       8.23    0.0014
       Days*Mois                    8     1.99862249      0.24982781       5.84    0.0002


Effect of incubation time on net N release for stable ‘Vlakplaas’- at 45 ° C               4
                                                       19:24 Thursday, November 25, 2008

                                        The GLM Procedure

                           Tukey's Studentized Range (HSD) Test for VI

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                  30
                            Error Mean Square                    0.04275
                            Critical Value of Studentized Range  4.10208
                            Minimum Significant Difference        0.2827


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

```
                      Tukey Grouping          Mean      N    Days

                                   A       3.86233      9    14

                                   B       3.29311      9    28

                              C    B       3.24967      9    7

                              C    B       3.15422      9    56

                              C            3.00378      9    1


Effect of incubation time on net N release for stable ‘Vlakplaas’- at 45 ° C            5
                                                  19:24 Thursday, November 25, 2008

                                    The GLM Procedure

                        Tukey's Studentized Range (HSD) Test for VI

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                  30
                        Error Mean Square                    0.04275
                        Critical Value of Studentized Range  3.48651
                       Minimum Significant Difference        0.1861




Means with the same letter are not significantly different.


                     Tukey Grouping          Mean      N    Mois

                                  A       3.48793     15    W2

                                  B       3.24513     15    W1

                                  B       3.20480     15    W3



Effect of incubation time on net N release for stable ‘Vlakplaas’- at 45 ° C            6
                                                  19:24 Thursday, November 25, 2008

                                    The GLM Procedure


                 Level of     Level of            --------------VI-------------
                 Days         Mois          N             Mean          Std Dev

                 1            W1            3       3.25233333       0.07950052
                 1            W2            3       2.99800000       0.00400000
                 1            W3            3       2.76100000       0.02116601
                 14           W1            3       3.90300000       0.07731106
                 14           W2            3       3.81766667       0.03066486
                 14           W3            3       3.86633333       0.07324161
                 28           W1            3       2.90500000       0.05483612
                 28           W2            3       3.75466667       0.02274496
                 28           W3            3       3.21966667       0.25032845
                 56           W1            3       3.05533333       0.05950070
                 56           W2            3       3.13000000       0.34393023
```

```
         56         W3        3      3.27733333      0.04072264
         7          W1        3      3.11000000      0.02787472
         7          W2        3      3.73933333      0.46262872
         7          W3        3      2.89966667      0.46658904


Effect of incubation time on net N release for stable ‘Vlakplaas’- at 45 ° C              7
                                                   19:24 Thursday, November 25, 2008

                                    The GLM Procedure
                                   Least Squares Means

                                                Standard                 LSMEAN
          Days          VI LSMEAN                  Error    Pr > |t|      Number

          1            3.00377778     0.06892019      <.0001           1
          14           3.86233333     0.06892019      <.0001           2
          28           3.29311111     0.06892019      <.0001           3
          56           3.15422222     0.06892019      <.0001           4
          7            3.24966667     0.06892019      <.0001           5



                            Least Squares Means for effect Days
                         Pr > |t| for H0: LSMean(i)=LSMean(j)

                                 Dependent Variable: VI

            i/j            1             2             3             4             5

               1                    <.0001        0.0058        0.1332        0.0172
               2        <.0001                    <.0001        <.0001        <.0001
               3        0.0058      <.0001                      0.1645        0.6590
               4        0.1332      <.0001        0.1645                      0.3353
               5        0.0172      <.0001        0.6590        0.3353


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



                                                Standard                 LSMEAN
          Mois          VI LSMEAN                  Error    Pr > |t|      Number

          W1           3.24513333     0.05338535      <.0001           1
          W2           3.48793333     0.05338535      <.0001           2
          W3           3.20480000     0.05338535      <.0001           3


                         Least Squares Means for effect Mois
                         Pr > |t| for H0: LSMean(i)=LSMean(j)

                                 Dependent Variable: VI

                          i/j            1             2             3

                             1                    0.0031        0.5971
                             2        0.0031                    0.0008
                             3        0.5971      0.0008
```

```
NOTE: To ensure overall protection level, only probabilities associated with pre-planned







Effect of incubation time on net N release for stable ‘Vlakplaas’- at 45 ° C                8
                                                       19:24 Thursday, November 25, 2008

                                     The GLM Procedure
                                    Least Squares Means

      comparisons should be used.


                                                  Standard                    LSMEAN
                     Days     Mois       VI LSMEAN          Error     Pr > |t|       Number

                     1        W1        3.25233333      0.11937327      <.0001            1
                     1        W2        2.99800000      0.11937327      <.0001            2
                     1        W3        2.76100000      0.11937327      <.0001            3
                     14       W1        3.90300000      0.11937327      <.0001            4
                     14       W2        3.81766667      0.11937327      <.0001            5
                     14       W3        3.86633333      0.11937327      <.0001            6
                     28       W1        2.90500000      0.11937327      <.0001            7
                     28       W2        3.75466667      0.11937327      <.0001            8
                     28       W3        3.21966667      0.11937327      <.0001            9
                     56       W1        3.05533333      0.11937327      <.0001           10
                     56       W2        3.13000000      0.11937327      <.0001           11
                     56       W3        3.27733333      0.11937327      <.0001           12
                     7        W1        3.11000000      0.11937327      <.0001           13
                     7        W2        3.73933333      0.11937327      <.0001           14
                     7        W3        2.89966667      0.11937327      <.0001           15



                           Least Squares Means for effect Days*Mois
                              Pr > |t| for H0: LSMean(i)=LSMean(j)

                                   Dependent Variable: VI

    i/j           1           2           3           4           5           6           7           8

      1                  0.1424      0.0067      0.0006      0.0022      0.0010      0.0484      0.0057
      2      0.1424                  0.1706      <.0001      <.0001      <.0001      0.5858      0.0001
      3      0.0067      0.1706                  <.0001      <.0001      <.0001      0.4004      <.0001
      4      0.0006      <.0001      <.0001                  0.6169      0.8295      <.0001      0.3866
      5      0.0022      <.0001      <.0001      0.6169                  0.7751      <.0001      0.7116
      6      0.0010      <.0001      <.0001      0.8295      0.7751                  <.0001      0.5134
      7      0.0484      0.5858      0.4004      <.0001      <.0001      <.0001                  <.0001
      8      0.0057      0.0001      <.0001      0.3866      0.7116      0.5134      <.0001
      9      0.8479      0.1991      0.0108      0.0003      0.0013      0.0006      0.0721      0.0035
     10      0.2524      0.7365      0.0915      <.0001      <.0001      <.0001      0.3803      0.0003
     11      0.4743      0.4404      0.0368      <.0001      0.0003      0.0001      0.1926      0.0009
     12      0.8833      0.1084      0.0046      0.0009      0.0032      0.0015      0.0352      0.0083
     13      0.4058      0.5121      0.0474      <.0001      0.0002      0.0001      0.2341      0.0006
     14      0.0072      0.0001      <.0001      0.3401      0.6460      0.4577      <.0001      0.9282
     15      0.0453      0.5646      0.4179      <.0001      <.0001      <.0001      0.9750      <.0001
```

```
Effect of incubation time on net N release for stable ‘Vlakplaas’- at 45 ° C              9
                                                     19:24 Thursday, November 25, 2008

                                      The GLM Procedure
                                     Least Squares Means

                            Least Squares Means for effect Days*Mois
                              Pr > |t| for H0: LSMean(i)=LSMean(j)

                                    Dependent Variable: VI

  i/j           9            10            11            12            13            14            15

     1     0.8479        0.2524        0.4743        0.8833        0.4058        0.0072        0.0453
     2     0.1991        0.7365        0.4404        0.1084        0.5121        0.0001        0.5646
     3     0.0108        0.0915        0.0368        0.0046        0.0474        <.0001        0.4179
     4     0.0003        <.0001        <.0001        0.0009        <.0001        0.3401        <.0001
     5     0.0013        <.0001        0.0003        0.0032        0.0002        0.6460        <.0001
     6     0.0006        <.0001        0.0001        0.0015        0.0001        0.4577        <.0001
     7     0.0721        0.3803        0.1926        0.0352        0.2341        <.0001        0.9750
     8     0.0035        0.0003        0.0009        0.0083        0.0006        0.9282        <.0001
     9                   0.3381        0.5992        0.7350        0.5209        0.0044        0.0677
    10     0.3381                      0.6615        0.1985        0.7483        0.0003        0.3638
    11     0.5992        0.6615                      0.3897        0.9065        0.0011        0.1826
    12     0.7350        0.1985        0.3897                      0.3295        0.0103        0.0329
    13     0.5209        0.7483        0.9065        0.3295                      0.0008        0.2224
    14     0.0044        0.0003        0.0011        0.0103        0.0008                      <.0001
    15     0.0677        0.3638        0.1826        0.0329        0.2224        <.0001

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

```
Effect of incubation time on net N release for stable 'Vlakplaas'- at 45 °C 10
                                                                 19:24 Thursday, November 25, 2008

                                        The UNIVARIATE Procedure
                                          Variable:  VI  (N03)

                                                Moments

                        N                         45    Sum Weights                 45
                        Mean              3.31262222    Sum Observations       149.068
                        Std Deviation     0.42178874    Variance            0.17790574
                        Skewness          0.30701524    Kurtosis            -0.8878836
                        Uncorrected SS    501.633822    Corrected SS        7.82785258
                        Coeff Variation   12.7327751    Std Error Mean      0.06287655


                                       Basic Statistical Measures

                              Location                    Variability

                           Mean     3.312622     Std Deviation            0.42179
                           Median   3.252000     Variance                 0.17791
                           Mode     3.259000     Range                    1.76700
                                                 Interquartile Range      0.75400

                                       Tests for Location: Mu0=0

                             Test           -Statistic-    -----p Value------

                             Student's t    t  52.68454    Pr > |t|    <.0001
                             Sign           M      22.5    Pr >= |M|   <.0001
                             Signed Rank    S     517.5    Pr >= |S|   <.0001


                                          Tests for Normality

                          Test                  --Statistic---    -----p Value------

                          Shapiro-Wilk          W     0.952973    Pr < W      0.0659
                          Kolmogorov-Smirnov    D     0.131144    Pr > D      0.0500
                          Cramer-von Mises      W-Sq  0.150683    Pr > W-Sq   0.0228
                          Anderson-Darling      A-Sq  0.907237    Pr > A-Sq   0.0203


                                        Quantiles (Definition 5)

                                         Quantile      Estimate

                                         100% Max         4.264
                                         99%              4.264
                                         95%              3.947
                                         90%              3.848
                                         75% Q3           3.748
                                         50% Median       3.252

Effect of incubation time on net N release for stable 'Vlakplaas'- at 45 °C            11
                                                         19:24 Thursday, November 25, 2008

                                        The UNIVARIATE Procedure
                                          Variable:  VI  (N03)

                                        Quantiles (Definition 5)

                                         Quantile      Estimate

                                         25% Q1           2.994
```

```
                                  10%               2.791
                                  5%                2.753
                                  1%                2.497
                                  0% Min            2.497


                                   Extreme Observations

                             -----Lowest----        ----Highest----

                              Value      Obs         Value      Obs

                              2.497       17         3.848       27
                              2.745        9         3.872       19
                              2.753        8         3.947       25
                              2.785        7         3.991       20
                              2.791       18         4.264       13

                                     Frequency Counts

                  Percents                           Percents                           Percents
   Value Count  Cell   Cum          Value Count  Cell   Cum          Value Count  Cell   Cum

   2.497     1   2.2   2.2          3.055     1   2.2  35.6          3.564     1   2.2  71.1
   2.745     1   2.2   4.4          3.087     1   2.2  37.8          3.736     1   2.2  73.3
   2.753     1   2.2   6.7          3.102     1   2.2  40.0          3.748     1   2.2  75.6
   2.785     1   2.2   8.9          3.115     1   2.2  42.2          3.780     1   2.2  77.8
   2.791     1   2.2  11.1          3.141     1   2.2  44.4          3.784     1   2.2  80.0
   2.854     1   2.2  13.3          3.173     1   2.2  46.7          3.804     1   2.2  82.2
   2.898     1   2.2  15.6          3.249     1   2.2  48.9          3.825     1   2.2  84.4
   2.906     1   2.2  17.8          3.252     1   2.2  51.1          3.844     1   2.2  86.7
   2.952     1   2.2  20.0          3.259     2   4.4  55.6          3.846     1   2.2  88.9
   2.958     1   2.2  22.2          3.324     1   2.2  57.8          3.848     1   2.2  91.1
   2.963     1   2.2  24.4          3.332     1   2.2  60.0          3.872     1   2.2  93.3
   2.994     1   2.2  26.7          3.390     1   2.2  62.2          3.947     1   2.2  95.6
   2.996     1   2.2  28.9          3.411     1   2.2  64.4          3.991     1   2.2  97.8
   2.998     1   2.2  31.1          3.448     1   2.2  66.7          4.264     1   2.2 100.0
   3.002     1   2.2  33.3          3.526     1   2.2  68.9
```

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Effect of incubation time on net N release for stable ‘Vlakplaas’- at 45 ° C 12
                                                 19:24 Thursday, November 25, 2008

                             The UNIVARIATE Procedure
                               Variable:  VI  (N03)

                     Stem Leaf                     #             Boxplot
                       42 6                        1                |
                       41                                           |
                       40                                           |
                       39 59                       2                |
                       38 024557                   6                |
                       37 4588                     4             +-----+
                       36                                        |     |
                       35 36                       2             |     |
                       34 15                       2             |     |
                       33 239                      3             |  +  |
                       32 5566                     4             *-----*
                       31 0247                     4             |     |
                       30 00069                    5             |     |
                       29 015669                   6             +-----+
                       28 5                        1                |
                       27 4589                     4                |
```

```
    26                                           |
    25 0                       1                 |
    24
       ----+----+----+----+
   Multiply Stem.Leaf by 10**-1


                   Normal Probability Plot
4.25+                                               *+
    |                                           +++
    |                                         ++
    |                                      ++* *
    |                                 ******
    |                              *** ++
    |                               +++
    |                             *+
    |                           **
3.35+                        ++**
    |                      +***
    |                   ++***
    |                 ++***
    |              ******
    |            **+
    |      * * **
    |        ++
    |     +++
2.45+   *++
     +----+----+----+----+----+----+----+----+----+----+
         -2        -1         0        +1        +2
```

A.$_{3.1}$ Effect of incubation time on N0$_3$ release-Temperature 2

```
                                                                 1
                              19:27 Thursday, November 25, 2008

          Obs    Days    Mois    Rep       VI

            1      1      W1      1     0.138
            2      1      W1      2     0.130
            3      1      W1      3     0.345
            4      1      W2      1     0.140
            5      1      W2      2     0.307
            6      1      W2      3     0.192
            7      1      W3      1     0.286
            8      1      W3      2     0.258
            9      1      W3      3     0.262
           10      7      W1      1     0.820
           11      7      W1      2     0.932
           12      7      W1      3     1.099
           13      7      W2      1     0.680
           14      7      W2      2     0.636
           15      7      W2      3     0.596
           16      7      W3      1     0.762
           17      7      W3      2     0.875
           18      7      W3      3     0.835
           19     14      W1      1     1.097
           20     14      W1      2     1.514
           21     14      W1      3     1.570
           22     14      W2      1     1.295
           23     14      W2      2     1.179
           24     14      W2      3     0.845
           25     14      W3      1     0.930
           26     14      W3      2     1.363
           27     14      W3      3     0.950
           28     28      W1      1     2.792
           29     28      W1      2     2.713
           30     28      W1      3     2.769
```

```
                                31     28      W2      1     2.612
                                32     28      W2      2     2.533
                                33     28      W2      3     2.477
                                34     28      W3      1     2.616
                                35     28      W3      2     2.743
                                36     28      W3      3     2.465
                                37     56      W1      1     2.944
                                38     56      W1      2     2.992
                                39     56      W1      3     2.864
                                40     56      W2      1     2.642
                                41     56      W2      2     2.602
                                42     56      W2      3     2.638
                                43     56      W3      1     2.410
                                44     56      W3      2     2.609
                                45     56      W3      3     2.530
```

```
Effect of incubation time on N03 release-Temperature 2                             2
                                                   19:27 Thursday, November 25, 2008

                                        The GLM Procedure

                                     Class Level Information

Class        Levels  Values

Days              5  1 14 28 56 7

Mois              3  W1 W2 W3

Rep               3  1 2 3

VI               45  0.13 0.138 0.14 0.192 0.258 0.262 0.286 0.307 0.345 0.596 0.636 0.68 0.762
                     0.82 0.835 0.845 0.875 0.93 0.932 0.95 1.097 1.099 1.179 1.295 1.363 1.514
                     1.57 2.41 2.465 2.477 2.53 2.533 2.602 2.609 2.612 2.616 2.638 2.642 2.713
                     2.743 2.769 2.792 2.864 2.944 2.992


                                   Number of observations    45

Effect of incubation time on N03 release-Temperature 2                              3
                                                    19:27 Thursday, November 25, 2008


                          The GLM Procedure


Dependent Variable: VI   N03

                                               Sum of
       Source                      DF         Squares     Mean Square    F Value    Pr > F

       Model                       14     44.83849191      3.20274942     178.86    <.0001

       Error                       30      0.53719467      0.01790649

       Corrected Total             44     45.37568658

                        R-Square     Coeff Var      Root MSE      VI Mean

                        0.988161      8.857106      0.133815     1.510822
```

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

       Days                         4     44.14732947     11.03683237     616.36    <.0001
       Mois                         2      0.43200111      0.21600056      12.06    0.0001
       Days*Mois                    8      0.25916133      0.03239517       1.81    0.1145


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

       Days                         4     44.14732947     11.03683237     616.36    <.0001
       Mois                         2      0.43200111      0.21600056      12.06    0.0001
       Days*Mois                    8      0.25916133      0.03239517       1.81    0.1145



Effect of incubation time on N03 release-Temperature 2                               4
                                                    19:27 Thursday, November 25, 2008

                                          The GLM Procedure

                             Tukey's Studentized Range (HSD) Test for VI

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                    30
                             Error Mean Square                     0.017906
                             Critical Value of Studentized Range    4.10208
                             Minimum Significant Difference           0.183


                      Means with the same letter are not significantly different.


                       Tukey Grouping          Mean      N    Days

                                    A       2.69233      9    56

                                    A       2.63556      9    28

                                    B       1.19367      9    14

                                    C       0.80389      9    7

                                    D       0.22867      9    1




Effect of incubation time on N03 release-Temperature 2                             5
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                                         19:27 Thursday, November 25, 2008

                                 The GLM Procedure

                     Tukey's Studentized Range (HSD) Test for VI

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                  30
                     Error Mean Square                   0.017906
                     Critical Value of Studentized Range  3.48651
                     Minimum Significant Difference        0.1205




                  Means with the same letter are not significantly different.


                Tukey Grouping          Mean      N    Mois

                             A       1.64793     15    W1

                             B       1.45960     15    W3

                             B       1.42493     15    W2

Effect of incubation time on N03 release-Temperature 2                               6
                                          19:27 Thursday, November 25, 2008

                                 The GLM Procedure


                  Level of      Level of          --------------VI-------------
                  Days          Mois       N              Mean          Std Dev

                  1             W1         3        0.20433333       0.12188656
                  1             W2         3        0.21300000       0.08545759
                  1             W3         3        0.26866667       0.01514376
                  14            W1         3        1.39366667       0.25844213
                  14            W2         3        1.10633333       0.23363504
                  14            W3         3        1.08100000       0.24442381
                  28            W1         3        2.75800000       0.04063250
                  28            W2         3        2.54066667       0.06782576
                  28            W3         3        2.60800000       0.13917255
                  56            W1         3        2.93333333       0.06466323
                  56            W2         3        2.62733333       0.02203028
                  56            W3         3        2.51633333       0.10020146
                  7             W1         3        0.95033333       0.14040062
                  7             W2         3        0.63733333       0.04201587
                  7             W3         3        0.82400000       0.05729747


Effect of incubation time on N03 release-Temperature 2                                  7
                                             19:27 Thursday, November 25, 2008

                                 The GLM Procedure
                                Least Squares Means

                                        Standard                      LSMEAN
                   Days       VI LSMEAN        Error    Pr > |t|      Number

                   1         0.22866667   0.04460504      <.0001           1
                   14        1.19366667   0.04460504      <.0001           2
                   28        2.63555556   0.04460504      <.0001           3
                   56        2.69233333   0.04460504      <.0001           4
```

```
                     7         0.80388889      0.04460504      <.0001          5



                                 Least Squares Means for effect Days
                              Pr > |t| for H0: LSMean(i)=LSMean(j)

                                     Dependent Variable: VI

                i/j              1             2             3             4             5

                   1                      <.0001        <.0001        <.0001        <.0001
                   2        <.0001                      <.0001        <.0001        <.0001
                   3        <.0001        <.0001                      0.3752        <.0001
                   4        <.0001        <.0001        0.3752                      <.0001
                   5        <.0001        <.0001        <.0001        <.0001

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

```
                                               Standard                  LSMEAN
                   Mois       VI LSMEAN           Error    Pr > |t|      Number

                   W1        1.64793333      0.03455092      <.0001           1
                   W2        1.42493333      0.03455092      <.0001           2
                   W3        1.45960000      0.03455092      <.0001           3


                               Least Squares Means for effect Mois
                               Pr > |t| for H0: LSMean(i)=LSMean(j)

                                      Dependent Variable: VI

                          i/j              1             2             3

                             1                      <.0001        0.0006
                             2       <.0001                       0.4835
                             3       0.0006        0.4835


NOTE: To ensure overall protection level, only probabilities associated with pre-planned



Effect of incubation time on N03 release-Temperature 2                              8
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                                        The GLM Procedure
                                       Least Squares Means

      comparisons should be used.


                                                   Standard                  LSMEAN
               Days    Mois       VI LSMEAN           Error    Pr > |t|      Number

               1       W1        0.20433333      0.07725820      0.0129           1
               1       W2        0.21300000      0.07725820      0.0098           2
               1       W3        0.26866667      0.07725820      0.0016           3
               14      W1        1.39366667      0.07725820      <.0001           4
               14      W2        1.10633333      0.07725820      <.0001           5
               14      W3        1.08100000      0.07725820      <.0001           6
               28      W1        2.75800000      0.07725820      <.0001           7
               28      W2        2.54066667      0.07725820      <.0001           8
               28      W3        2.60800000      0.07725820      <.0001           9
               56      W1        2.93333333      0.07725820      <.0001          10
               56      W2        2.62733333      0.07725820      <.0001          11
               56      W3        2.51633333      0.07725820      <.0001          12
               7       W1        0.95033333      0.07725820      <.0001          13
               7       W2        0.63733333      0.07725820      <.0001          14
               7       W3        0.82400000      0.07725820      <.0001          15




                             Least Squares Means for effect Days*Mois
                               Pr > |t| for H0: LSMean(i)=LSMean(j)

                                      Dependent Variable: VI

   i/j           1          2          3          4          5          6          7          8

      1                0.9373     0.5604     <.0001     <.0001     <.0001     <.0001     <.0001
      2     0.9373                0.6141     <.0001     <.0001     <.0001     <.0001     <.0001
      3     0.5604     0.6141                <.0001     <.0001     <.0001     <.0001     <.0001
      4     <.0001     <.0001     <.0001                0.0133     0.0076     <.0001     <.0001
      5     <.0001     <.0001     <.0001     0.0133                0.8182     <.0001     <.0001
```

```
      6     <.0001     <.0001     <.0001     0.0076     0.8182                <.0001     <.0001
      7     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001                0.0559
      8     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     0.0559
      9     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     0.1800     0.5424
     10     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     0.1190     0.0011
     11     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     0.2411     0.4339
     12     <.0001     <.0001     <.0001     <.0001     <.0001     <.0001     0.0347     0.8253
     13     <.0001     <.0001     <.0001     0.0003     0.1637     0.2411     <.0001     <.0001
     14     0.0004     0.0005     0.0021     <.0001     0.0002     0.0003     <.0001     <.0001
     15     <.0001     <.0001     <.0001     <.0001     0.0149     0.0254     <.0001     <.0001


Effect of incubation time on N03 release-Temperature 2                                   9
                                                  19:27 Thursday, November 25, 2008

                                         The GLM Procedure
                                        Least Squares Means

                                Least Squares Means for effect Days*Mois
                                  Pr > |t| for H0: LSMean(i)=LSMean(j)

                                       Dependent Variable: VI

  i/j            9            10            11            12            13            14            15

     1      <.0001        <.0001        <.0001        <.0001        <.0001        0.0004        <.0001
     2      <.0001        <.0001        <.0001        <.0001        <.0001        0.0005        <.0001
     3      <.0001        <.0001        <.0001        <.0001        <.0001        0.0021        <.0001
     4      <.0001        <.0001        <.0001        <.0001        0.0003        <.0001        <.0001
     5      <.0001        <.0001        <.0001        <.0001        0.1637        0.0002        0.0149
     6      <.0001        <.0001        <.0001        <.0001        0.2411        0.0003        0.0254
     7      0.1800        0.1190        0.2411        0.0347        <.0001        <.0001        <.0001
     8      0.5424        0.0011        0.4339        0.8253        <.0001        <.0001        <.0001
     9                    0.0057        0.8607        0.4081        <.0001        <.0001        <.0001
    10      0.0057                      0.0088        0.0006        <.0001        <.0001        <.0001
    11      0.8607        0.0088                      0.3178        <.0001        <.0001        <.0001
    12      0.4081        0.0006        0.3178                      <.0001        <.0001        <.0001
    13      <.0001        <.0001        <.0001        <.0001                      0.0076        0.2567
    14      <.0001        <.0001        <.0001        <.0001        0.0076                      0.0979
    15      <.0001        <.0001        <.0001        <.0001        0.2567        0.0979


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


Effect of incubation time on N03 release-Temperature 2                                    10
                                                    19:27 Thursday, November 25, 2008

                                       The UNIVARIATE Procedure
                                         Variable:  VI  (N03)

                                               Moments

                N                          45    Sum Weights                 45
                Mean               1.51082222    Sum Observations        67.987
                Std Deviation      1.01551248    Variance             1.0312656
                Skewness            0.1281016    Kurtosis              -1.65573
                Uncorrected SS     148.091957    Corrected SS        45.3756866
                Coeff Variation    67.2158821    Std Error Mean      0.15138366


                                      Basic Statistical Measures

                        Location                        Variability

                    Mean     1.510822     Std Deviation             1.01551
                    Median   1.179000     Variance                  1.03127
                    Mode      .           Range                     2.86200
```

```
                           Interquartile Range      1.92900


                   Tests for Location: Mu0=0

        Test           -Statistic-    -----p Value------

        Student's t    t  9.980088    Pr > |t|    <.0001
        Sign           M      22.5    Pr >= |M|   <.0001
        Signed Rank    S     517.5    Pr >= |S|   <.0001


                      Tests for Normality

   Test                  --Statistic---    -----p Value------

   Shapiro-Wilk          W     0.864314    Pr < W     <0.0001
   Kolmogorov-Smirnov    D     0.212041    Pr > D     <0.0100
   Cramer-von Mises      W-Sq  0.391966    Pr > W-Sq  <0.0050
   Anderson-Darling      A-Sq  2.378979    Pr > A-Sq  <0.0050


                    Quantiles (Definition 5)

                     Quantile      Estimate

                     100% Max         2.992
                     99%              2.992
                     95%              2.864
                     90%              2.769
                     75% Q3           2.609
                     50% Median       1.179



Effect of incubation time on N03 release-Temperature 2                               11
                                                         19:27 Thursday, November 25, 2008

                    The UNIVARIATE Procedure
                      Variable:  VI  (N03)

                    Quantiles (Definition 5)

                     Quantile      Estimate

                     25% Q1           0.680
                     10%              0.258
                     5%               0.140
                     1%               0.130
                     0% Min           0.130




                      Extreme Observations

              -----Lowest----        ----Highest----

               Value      Obs         Value      Obs

               0.130        2         2.769       30
               0.138        1         2.792       28
               0.140        4         2.864       39
               0.192        6         2.944       37
               0.258        8         2.992       38



                        Frequency Counts
```

```
                  Percents                          Percents                          Percents
    Value Count  Cell    Cum           Value Count  Cell    Cum           Value Count  Cell    Cum

    0.130     1   2.2   2.2           0.845     1   2.2  35.6           2.530     1   2.2  68.9
    0.138     1   2.2   4.4           0.875     1   2.2  37.8           2.533     1   2.2  71.1
    0.140     1   2.2   6.7           0.930     1   2.2  40.0           2.602     1   2.2  73.3
    0.192     1   2.2   8.9           0.932     1   2.2  42.2           2.609     1   2.2  75.6
    0.258     1   2.2  11.1           0.950     1   2.2  44.4           2.612     1   2.2  77.8
    0.262     1   2.2  13.3           1.097     1   2.2  46.7           2.616     1   2.2  80.0
    0.286     1   2.2  15.6           1.099     1   2.2  48.9           2.638     1   2.2  82.2
    0.307     1   2.2  17.8           1.179     1   2.2  51.1           2.642     1   2.2  84.4
    0.345     1   2.2  20.0           1.295     1   2.2  53.3           2.713     1   2.2  86.7
    0.596     1   2.2  22.2           1.363     1   2.2  55.6           2.743     1   2.2  88.9
    0.636     1   2.2  24.4           1.514     1   2.2  57.8           2.769     1   2.2  91.1
    0.680     1   2.2  26.7           1.570     1   2.2  60.0           2.792     1   2.2  93.3
    0.762     1   2.2  28.9           2.410     1   2.2  62.2           2.864     1   2.2  95.6
    0.820     1   2.2  31.1           2.465     1   2.2  64.4           2.944     1   2.2  97.8
    0.835     1   2.2  33.3           2.477     1   2.2  66.7           2.992     1   2.2 100.0
```

```
Effect of incubation time on N03 release-Temperature 2                             12
                                                    19:27 Thursday, November 25, 2008

                                     The UNIVARIATE Procedure
                                       Variable:  VI  (N03)

                         Stem Leaf                     #             Boxplot
                           28 649                      3                |
                           26 0112441479              10             +-----+
                           24 16833                    5             |     |
                           22                                        |     |
                           20                                        |     |
                           18                                        |     |
                           16                                        |     |
                           14 17                       2             |  +  |
                           12 06                       2             |     |
                           10 008                      3             *-----*
                            8 2448335                  7             |     |
                            6 0486                     4             +-----+
                            4                                           |
                            2 66914                    5                |
                            0 3449                     4                |
                              ----+----+----+----+
                          Multiply Stem.Leaf by 10**-1
```

```
Normal Probability Plot
                        2.9+                                      ++ * *   *
                           |                               *********
                           |                            ***   ++
                           |                                ++
                           |                              ++
                           |                            ++
                           |                          ++
                        1.5+                        ++ *
                           |                      ++  *
                           |                    ++  **
                           |                   +****
                           |                 +**
                           |               ++*
                           |            *****
                        0.1+   *   * * *+
                            +----+----+----+----+----+----+----+----+----+----+
                                -2        -1         0        +1        +2
```