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Nitrogen mineralization in a sludge-amended sandy clay loam in South Africa

Kvävemineralisering i en sydafrikansk jord gödslad med rötslam

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Abstract

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Sewage sludge is a solid, semi-solid or liquid byproduct produced by waste water treatment plants. It contains both compounds of agricultural value (e.g. organic material, nitrogen, phosphorous, potassium and to a lesser extent calcium, sulphur and magnesium), and pollutants (e.g. heavy metals, pathogens and toxic organics). The quality of sludge is dependent on its origins, i.e. whether it is domestic or industrial. Sludge can be disposed in many ways, e.g. through land application, incineration and land filling. Generally the least cost option, land application, has become the most common disposal method in many countries. Sludge production has increased dramatically due to population growth and increased indutrialization and it is very important to reduce and utilize the digested sewage sludge and to minimize its environmental impact. One way to achieve this is to apply sludge on farm land. Land application has many advantages and is expected to become the dominant disposal method in the future. This is especially true for small communities which have high quality sludge in terms of lower concentrations of chemical pollutants. In sludge, N is in organic form but is mineralized to NH_4^+ and NO_3^- and therefore becomes plant available.

The main aim of the present study was to compare N mineralization between two treatments (sludge amended soil (Sludge) and fertilization with ammonium nitrate (AN)) and a control (C) in a incubation experiment. A second aim was to quantify the effect of the treatments on soil pH. In both Sludge and AN, equal amounts of total N in organic and inorganic form were added. The upper limit for the amount of sludge farmers are allowed to apply on arable land in South Africa is 8 tonnes dry matter ha^{-1} $year^{-1}$ and this was also the amount used in this incubation study.

In all three treatments, mineral N changed significantly during the incubation. For Sludge and C, mineral N increased with time while it decreased for AN. This decrease in inorganic N was unexpected and might be due to gaseous N losses. The pH did not change significantly with time for Sludge and C but decreased significantly for AN as the soil became more and more acidified due to proton release during nitrification.

Referat

Kväve mineraliserings i en syd-afrikansk jord gödslad med rötslam.

Anna-Karin Mellin

Rötslam är den slutprodukt som bildas när vatten och avlopp från hushåll och industrier renats vid reningsverken. Slammet innehåller både nyttiga substanser som organiskt material, kväve, fosfor, kalium och i en mindre utsträckning kalcium, svavel och magnesium men även föroreningar som tung metaller, sjukdomsalstrande organismer och oorganiska substanser. Alla dessa egenskaper är beroende av rötslammets ursprung.

Rötslam kan användas bl.a. inom jordbruket som näringskälla, till uppfyllning av landområden eller förbrännas. Problem såsom befolkningsökning och ökad industrialisering gör att alternativ till deponering måste hittas och tack vare de många fördelarna hos rötslam så ökar nu efterfrågan inom jordbrukat. Det organiska kvävet i rötslammet mineraliseras med hjälp av mikroorganismerna i jorden till oorganiskt kväve som då blir växttillgängligt.

Syftet med denna studie var att undersöka mineraliseringen av kväve i jorden för två olika behandlingar samt en kontroll där lika mängd total N tillsatts båda. I en av behandlingarna har rötslam tillsats och i den andra ammoniumnitrat. Även pH jämförs mellan behandlingarna. I Sydafrika får 8 ton torrmassa av rötslam tillföras åkermarken varje år. Denna mängd rötslam har använts även i denna studie.

Resultaten visar att i rötslamsbehandlingen skedde en positiv nettomineralisering, dvs mängden oorganiskt kväve ökade med tiden, medan den minskade i ammoniumnitrat-behandlingen. Denna minskning kan bero på att miljön förändrades i jorden så att N_2, N_2O och NO producerades.

I den först nämnda behandlingen så ökade pH med tiden även om ökningen här inte var signifikant. I den tredje behandlingen så blev jorden surare med tiden på grund av oxidationen av ammonium till nitrat varvid det bildas ett överskott av protoner.

PREFACE

This thesis corresponds to 20 credits on D-level in Soil Science to complete my four-year Master of Science degree in Natural Resources at the Swedish University of Agriculture. The thesis was written for the Department of Plant Production and Soil Science at the University of Pretoria, South Africa and the Department of Soil Science, Section of Soil Fertility and Plant Nutrition, at the Swedish University of Agricultural Science (SLU).

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Anna-Karin Mellin

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1. INTRODUCTION

1.1. AIMS

This study consists of a literature review and an incubation experiment. The aim of the literature study was to provide an introduction to sludge, how it is disposed of and the advantages and disadvantages of using sludge in the field, and also to provide a short introduction to nitrogen (N) transformations in soil, since soils amended with sludge often have a high N-mineralization potential.

The first aim of the incubation experiment was to compare the N release from a slow N source (sludge) with that from a 100% bio-available N source (ammonium nitrate) at different water potentials. The initial N content of sludge is distributed between inorganic (direct bio-available N) and organic (non bio-available N) forms and the aim was to quantify the amount of organic N that is mineralized under controlled conditions. The mineralization was measured for the drying zone of soil amended with sewage sludge and for the same soil fertilized with an equivalent amount of inorganic N. The potentials used in the incubation corresponded to water contents below field capacity (0.1-0.3 bar) which is the amount of water that is retained by a soil after internal drainage (Hillel, 1980).

The second aim was to measure how pH changed with time during the incubations at different water potentials.

1.2. DELIMITATION

The study only included sewage sludge from one waste water treatment plant (WWTP). However, it would be beneficial to examine sludge from several different WWTP as the substances in sludge can differ depending on its origin, e.g. industrial or municipal WWTP.

2. LITERATURE STUDY

2.1. SLUDGE INTRODUCTION

Sewage sludge is a solid, semi-solid or liquid byproduct produced by both domestic and industrial wastewater treatment plants. Disposal of sewage sludge has become an increasing problem and it is very important to reduce and utilize the wastes and to minimize their environmental impact (Novella, 1991; Palmer, 1991; Rutgers, 2005)

Most sewage sludge is disposed of through land application, land filling, lagooning, co-disposal with garbage, composting, ocean disposal or incineration (Ekama, 1993a). The most cost effective and most frequently used method of disposal for many years has been burial in landfills, but new regulations now make this practice much more expensive than in the past (ACES, 2005). In some parts of the world the growing trend is land application of sludge. Land application is the controlled spreading of sewage sludge onto or into the soil surface. In South Africa, it is by far the most popular way of disposing of sludge and the reasons are mainly:

1) Awareness of the usefulness of sewage sludge as an organic fertilizer. When treated properly and when certain industrial contaminants are restricted from entering the incoming sludge, the resultant sewage sludge can be a relatively risk-free, low cost, organic fertilizer to supplement conventional inorganic fertilizers and can be of significant value for agricultural and horticultural purposes (Hensler et al., 1970; Palmer, 1991; Ekama, 1993a; WHO, 1995; WRC, 1997).

2) Awareness of the usefulness of sewage sludge as a soil conditioner. When sludge (organic matter) is applied, the soil structure improves, which increases the stability of the soil aggregates and increases the water-holding capacity of the soil.

3) Cost effectiveness. Due to its nutrient value to the soil and its better economy as a disposal option, an application of sewage sludge would reduce N and P inputs and therefore reduce agricultural input costs.

4) Since ocean disposal of sludge is no longer permissible e.g. in Europe, land application is encouraged (European Community Directive 86/278/EEC, directive specific for sewage sludge, implemented 1989).

2.2. SLUDGE DISPOSAL

2.2.1. Europe and USA

In Europe, 28% of the 6.6 million tonnes of dry mass sewage sludge produced in 1992 was disposed through land application, while 43% was land filled, 10% incinerated and 9% disposed of by other methods (Davis, 1992). According to Davis (cit. Lötter & Pitman, 1997), farmers in the UK receive the sludge free on a voluntary basis and only sometimes bear the cost of spreading the material. Germany, France, the UK and Italy are Europe’s largest sludge producers, contributing 37.7%, 19.5%, 16.2% and 12.1% of the total annual tonnage respectively and using 25%, 50%, 51% and 34% of their sludge production respectively in agriculture (Ekama, 1993a).

In Sweden, about 45 000 tonnes (20%) of a total annual production of 220 000 tonnes (dry mass) of sewage sludge is used in agriculture. The rest is used for other purposes or disposed of in landfills (Naturvårdsverket, 2002).

In the USA (Iowa), farmers receive the sludge by injector trucks which inject the sludge into the soil, but before a farmer can take part in this system, his farm is carefully evaluated (Lötter & Pitman, 1997). The injector technique reduces the nitrogen loss and also bad odours.

2.2.2. South Africa

According to Smith & Vasiloudis (1989), over 55% of South Africa’s sludge was disposed in landfills, lagoons and other dedicated land sites located near urban areas at that time. Many of these sites were approaching the end of their lifespan, and the pressure on towns and cities to develop safe and feasible alternative practices for sludge management and disposal was increasing (Page et al., 1987). The amount of sludge was also increasing because of population growth and increasing industrialization and therefore the pressure on sewage disposal systems was expected become critical (Korentaljer, 1991). One way to accommodate this increase was to begin disposing of sludge on farms. The main motivation for utilization of sludge in agriculture is the fact that it appeared to be a cheap disposal option (Palmer, 1989).

Because of the advantages of land application, both environmentally and economically, the percentage of sludge disposed through land application has already increased in South Africa and this trend is expected to continue. This is especially true for small communities which have high quality sludge in terms of lower concentrations of chemical pollutants, and are located in areas where farmland is readily accessible.

2.3. NUTRIENTS IN SLUDGE

Studies have shown that application of sludge increase the yield of many plant species e.g. field crops, forage, ornamentals and to a lesser extent vegetables and conifers. This is mostly due to the increased supply of nutrients. Sludge can be considered as a low-grade fertilizer, as although its composition is extremely variable, it generally contains essential plant nutrients (Table 1).

Table 1 A*verage nutrient concentrations in sludge from 200 sources in eight states in the USA (ACES, 2005)*

Nutrient	**% of total dry matter**
Organic carbon, C	30.0
Total nitrogen, N	3.3
Total phosphorus, P (P_2O_5)	2.3 (5.27)
Potassium, K (K_2O)	0.3 (0.4)
Magnesium, Mg	0.4
Total sulphur, S	1.1
Calcium, Ca	3.9
Iron, Fe	0.4
Aluminium, Al	0.2

Through the use of sludge, the application of mineral fertilizers can be reduced. The nutrients in sludge are in a plant-unavailable organic form but through the process of mineralization in soil the organic form is slowly transformed to an inorganic form, which can be taken up by plants. The percentage of nutrients released per year after application are 40, 20, 10, 5 in years 1-4 and 2.5% in subsequent years. The release of nutrients is dependent on the climate and also the composition of the sludge (Palmer, 1991; Lötter & Pitman, 1997; WRC, 1997; ACES, 2005). However, if we compare the concentration of plant nutrients in sludge with commonly used inorganic fertilizers, the N and P concentrations in sludge are rather low. The concentration of N in sludge is around 10% of that in inorganic fertilizer (Korentaljer, 1991).

2.3.1. Sludge as a N fertilizer

The N present in sludge is in organic form and is mineralized slowly in soil. Therefore it may be beneficial to use sewage sludge as a slow release N source, e.g. in situations when the efficiency of N commercial fertilizers is limited due to high NO_3^- leaching losses, etc. (Korentaljer, 1991). On the other hand, this might be a problem due to a lack of synchronisation between crop demand and N mineralization (Dahlin et al., 2005).

2.3.2. Sludge as a P fertilizer

P is an expensive plant nutrient which is strongly bound to soil colloids and therefore less leachable than nitrate (White, 1981; Ag News, 2005). Studies indicate that sludge can be beneficially used in situations when the efficiency of commercial inorganic P sources is limited by soil factors, e.g. high soil P fixing capacity.

When the application rate of sludge is based on the crop N demand, the P supplied will be in excess of crop needs, which may lead to undesirable environmental consequences (Korentaljer, 1991).

2.4. SLUDGE AS A SOIL CONDITIONER

Soils in warm climates, like in South Africa, have often low organic matter content. This is due to intensive farming of the land, high microbial decomposition of the organic matter and also excessive use of mineral fertilizer that does not add any organic material to the soil (Stevenson, 1982). This decrease in organic matter in the soil may lead to the deterioration of soil physical properties.

When organic matter is applied to a sandy soil, its ability to hold water and the stability of the soil aggregates increase. In a clayey soil, water infiltration rate and hydraulic conductivity increase, root penetration is easier and surface water runoff and soil erosion are reduced. In order to give a significant effect, repeated applications of sludge over a period of several years may be required (Palmer, 1989; ACES, 2005).

Composting of sewage sludge results in a reduction in plant available nitrogen and composted sludge is therefore primarily used as a soil conditioner rather than a fertilizer since it is high in stabilized organic matter (Korentaljer, 1991; Rutgers, 2005).

2.5. DISADVANTAGES OF SLUDGE

Even if the sludge is treated properly, there are concerns regarding the consequences when sludge is applied to land. The biggest concern is food chain contamination. Humans can mainly be affected by consuming raw or semi-cooked vegetables or meat. To minimize such contamination, a time lag between the last application of sewage sludge and crop harvest is recommended. This time lag varies depending on crop species (Korentaljer, 1991).

In South Africa, hygiene quality norms for the use of sewage sludge in agriculture have been proposed (Oberholster, 1983) and current restrictions refer to numbers of organisms and bacteria, as well as heavy metal content (Department of National Health and Population Development, 1991). Bad odours can be satisfactorily dealt with by applying the sludge, usually in concentrated liquid form, below the soil surface during ploughing (Ekama, 1993a).

2.5.1. N and P contamination from sludge

Another concern is pollution of the groundwater. High application rates of sludge can lead to groundwater pollution by nitrate, which derives from nitrified ammonium released by the sludge. In Europe, high nitrate in groundwater is a particular problem, but it is hard to say if the reason is use of sludge or excessive use of inorganic fertilizer (Ekama, 1993a), since sludge is only used on a small scale in agriculture, the problem of high nitrate in groundwater is only local. To prevent accumulation of NO_3^- in the groundwater, proper estimations of soil available N and crop N needs are required (Korentaljer, 1991).

After application of excessive amounts of P, the environment may be negatively affected, with consequences such as accumulation of P in runoff and surface water reservoirs. In South Africa, surface water is the major source of drinking water, especially in rural areas, so this may then lead to a serious threat if the surface water becomes polluted. The accumulation of P in surface waters is the major factor contributing to an increased growth of algae (i.e. eutrophication), which may lead to a decrease in water quality. In this situation application rates should be based on the P requirements of the crop, and not its N requirements (Korentaljer, 1991) as practised e.g. in Sweden (Naturvårdsverket, 2002).

2.5.2. Heavy metals

There are two main exposure routes whereby humans can be endangered by heavy metals:

1) Intake of contaminated crops.
2) Consumption of organs (liver, kidneys) from animals grazed on land treated with sewage sludge (Ekama, 1993a).

The toxic heavy metals commonly present in sludge are in particular Cd, Hg, Pb, Zn, Mo, Ni, Cr and Cu (Corey et al., 1987; Korentaljer, 1991). These heavy metals sometimes build up in the soil to critical limits and are then assimilated into fruits and vegetables.

When toxic elements are applied to the soil through sludge, irrespective of the form in which they are applied, they may (i) pass through the soil unchanged, (ii) be in form of insoluble or soluble inorganic and organic compounds, (iii) be absorbed by soil colloids as cations, anions or molecules, (iv) be volatilized from the surface (Hg, As, Se), or (v) be taken up by plants (Page, 1974).

It is very expensive to remove these metals from the sludge and therefore it is important to prevent them entering the wastewater system, or otherwise an alternative to land application must be found (Novella, 1991). Cd appears to be the heavy metal posing the greatest threat because of its relatively high mobility in the soil (Korentaljer, 1991). Excessive intake of Cd through the diet has been shown to cause kidney failure in human beings (ACES, 2005). The greatest concern is in countries where polychlorinated biphenyls (PCBs) are used (WRC, 1997).

Table 2 shows the quality requirements set by two South African departments in respect to soil and sludge. Some limits are different and the two departments are trying to reach agreement on a single limit. Table 3 shows a comparison between South African and international heavy metal limits in the soil. We can see that the metal limits are stricter in South Africa than in the other countries.

Table 2 *South African health and agriculture heavy metal limits for soil and sludge (Lötter & Pitman, 1997)*

Trace Element	**Health Guide**		**Use in Agriculture**	
	Soil (mg kg^{-1})	Sludge (mg kg^{-1})	Soil (mg kg^{-1})	Sludge (mg kg^{-1})
Arsenic	2	15	20	80
Cadmium	2	20	3	20
Chromium	80	1750	100	1200
Copper	100	750	100	1200
Lead	56	400	100	1200
Mercury	0.5	10	2	25
Nickel	15	200	50	200
Selenium	2	15	5	-
Thallium	-	-	1	-
Uranium	-	-	5	-
Zinc	185	2750	300	3000
Boron	10	80	25	100
Cobalt	20	100	-	-
Molybdenum	2.3	25	-	-
F^- fluoride	50	400	200	-
CN^- cyanide	-	-	5	-

Table 3 *Comparison of South African and some international limit values for heavy metals in soil in mg kg^{-1} (Lötter & Pitman, 1997)*

Trace Element	**Maximum EEC* values**	**Probable UK values**	**SA limits for Soil**	
			Health	**Agriculture**
Zinc	300	300	185	300
Copper	140	135	100	100
Nickel	75	75	15	50
Cadmium	3	3	2	3
Lead	300	300	56	100
Chromium	-	600	80	100

* EEC (European Community Directive 86/278/EEC)

The main factor controlling the mobility of heavy metals is the pH of the soil, the characteristics of the applied sludge, sludge application rates, the crop species and the cultivar grown (Chaney et al., 1987; Korentaljer, 1991).

An important factor for many soils, especially in high rainfall areas where the soils can easily be acidified due to poor buffering capacity, is that the soil pH should be 6.5 or above, or otherwise the soils should be treated with lime (Page, 1974; Korentaljer, 1991; WRC, 1997). In the Southern United States, regulations require that the soil pH be adjusted to 6.0 or above before sludge is applied to the field. This requirement is an additional safeguard that limits metal uptake by plants and promotes optimum crop yields in most soils (Soil Facts, 2005).

A German study has concluded that the solubility of Zn, Ni and Cd in soils and sludge increases considerably with a decline in pH values, though the solubility for As, Se, B and Mo increases to some extent in more neutral and alkaline soils (Page, 1974; Korentaljer, 1991; Lötter & Pitman, 1997; WRC, 1997). A near-neutral pH renders heavy metals less soluble and therefore less available for plants.

2.5.3. Pathogens

The most common pathogens (disease-causing organisms) in sewage sludge are bacteria, protozoa, nematodes and viruses (Korentaljer, 1991). Sludge formed during wastewater treatment contains concentrated levels of these pathogens. If the sludge is not treated correctly, the pathogens within the sludge can pose a serious threat to humans and animals (Umass, 2005).

Pathways of exposure include:

1) Consumption of contaminated crops grown on sewage sludge-amended soil.
2) Consumption of contaminated milk or other food products from animals contaminated by grazing on sludge-treated pastures.
3) Ingestion of groundwater or surface water contaminated by runoff from nearby land application sites.
4) Consumption of inadequately cooked or uncooked contaminated fish.

In a warmer climate the risk of pathogen infections is much greater and therefore also the health hazards associated with the use of sewage sludge (Oberholster, 1983).

2.5.4. Toxic organics

The type and concentration of toxic organics found in sewage sludge depends on the composition of the wastewater, e.g. whether it is of urban or industrial origin (Page, 1974; Ekama, 1993a). Some examples of toxic organic compounds can be pesticides, PCBs, dioxins and phenolic compounds. The potential exposure route for toxic organics to humans is the same as for pathogens. To minimize the risk of contamination problems with toxic organics, a three-year waiting period before growing crops can be eaten raw is recommended (Ekama, 1993a).

PCBs are very toxic and persistent in the environment and have been demonstrated to cause cancer in humans and a number of serious non-cancer health effects in animals, including effects on the immune system, reproductive system and neurological system. The different health effects of PCBs may be interrelated, as alterations in one system may have significant implications for the other regulatory systems of the body (US EPA, 2005). Table 4 shows different contaminants in sludge, such as pathogens, nitrates and heavy metals, and ways to control them.

Table 4 *Potential contaminants in sludge and ways to control these (*ACES, 2005).

Contaminant	Potential concern	Solution
Pathogens: Bacteria and virus diseases	Human health	Disinfect sludge
Nitrates	Applying in excess of plant needs; excess application entering groundwater	Apply to achieve efficient crop utilization
Organics: Chlorinated hydrocarbon pesticides, polychlorinated biphenyls (PCBs), etc.	Health hazard if directly ingested by animals	Inject or disc into soil
Heavy metals:		
Copper, Zinc and Nickel	Accumulation in topsoil; toxic to plants at high levels	Reduce source of metal in sludge; apply according to soil loading limits; lime soil
Cadmium	Accumulation in topsoil; taken up by plant and accumulates in leafy material; accumulates in animal organs; human health	Reduce source of metal in sludge; apply according to soil loading limits; lime soil
Lead	Accumulation in topsoil: Potentially harmful if excessive amounts are ingested with soil particles by animals	Reduce source of metal in sludge; apply according to soil loading limits; lime soil
Mercury, Chromium, Selenium, Arsenic and Antimony	Little concern unless present in extremely high amounts	

2.6. NITROGEN

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

The global N cycle describes the movements of nitrogen in many forms between hydrosphere, lithosphere, atmosphere and biosphere. The nitrogen in the soil accounts for an important part of the global N cycle. In nature, N passes from one pool (soil, living organisms and atmosphere) to another through a variety of chemical and biological processes. Most of the nitrogen in soil is present in the form of complex organic molecules. Conversely, there is little present in the immediately usable nitrate or ammonium forms.

Gains in soil N occur though fixation of molecular N_2 by microorganisms and from rainwater by the return of NH_4^+ and NO_3^-. Losses occur though crop removal, leaching and volatilization. Of all the gases in the atmosphere, molecular N_2 comprises about 78%.

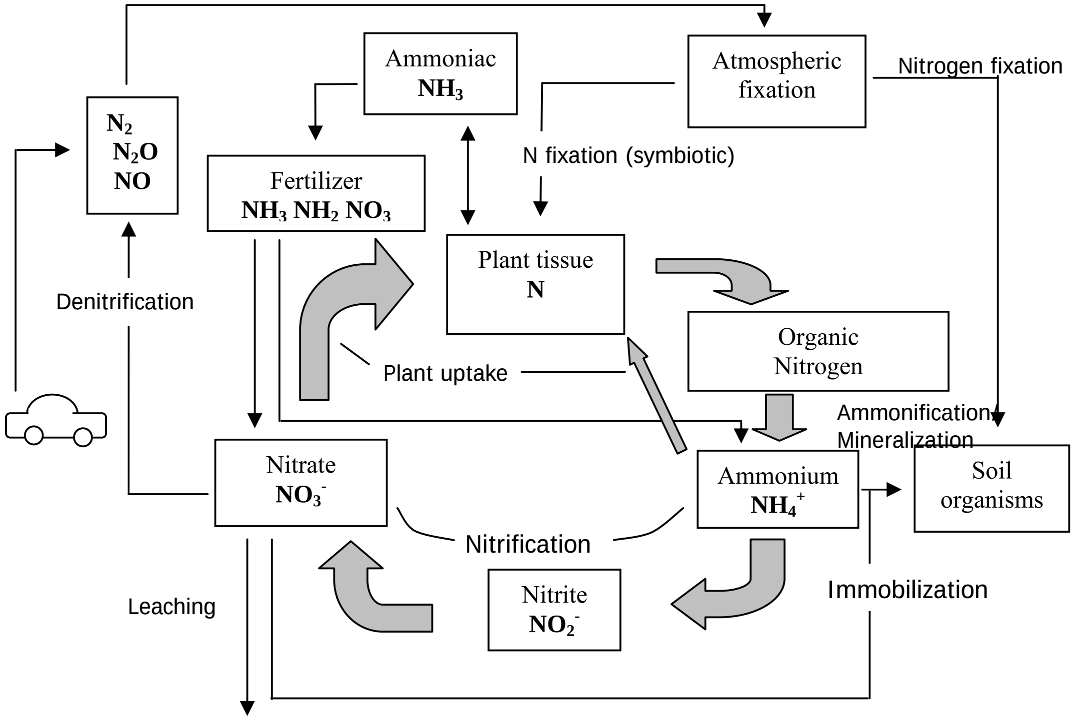

Figure 1 *A schematic presentation of the N cycle (Brady & Weil, 1999).*

2.7 DIFFERENT FORMS OF N PRESENT IN SOILS

2.7.1. Organic N

Organic nitrogen exists in materials formed from animal, human, and plant activities that produce manures, sewage waste, compost and decomposing roots or leaves. These organic products transform into organic soil material called humus. The remainder is in mineral form, including some fixed NH_4^+. Bacteria found in soils convert organic forms of nitrogen to inorganic forms that the plant can use.

2.7.2. Ammonia (NH_3) and Ammonium (NH_4^+)

In unfertilized soils, the greatest proportion of inorganic N comes from the mineralization of organic N and only a small proportion comes from weathering of minerals or is added to soil from precipitation. There are two forms of inorganic nitrogen, ammonium and nitrate, that can be directly assimilated by plants. Ammonium (NH_4^+) can be stored in the soil or can escape as ammonia gas (NH_3) into the Earth's atmosphere (NASA, 2005). NH_3 is the direct product of nitrogen fixation, see Section 2.8.1. In acid soils, NH_3 picks up a hydrogen ion (H^+) and forms NH_4^+,

which can be directly used by plants. At pH values > 9, NH_4^+ is volatilized and leaves the soil as ammonia gas.
Ammonium (NH_4^+) is one of the two forms of nitrogen that is available to plants, the other being NO_3^-. Ammonia gas reacts with water ions to form ammonium ions in the following equation (1):

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \qquad \text{(eq.1)}$$

2.7.3. Nitrite (NO_2^-)

Nitrite usually occurs in trace amounts in the soil. An accumulation of NO_2^- is of concern since it is a very mobile anion and highly toxic to microorganisms (Legg & Meisinger, 1982).

2.7.4. Nitrate (NO_3^-)

Nitrate is also a mobile anion but less reactive than NO_2^-. It is one of the most water soluble anions known and can cause serious surface water and groundwater problems. The amounts of NO_3^- in soils are normally in the range of a few to several hundred $\mu g\ g^{-1}$ (Falcone et al., 1963; Hooper, 1968 cit. Schmidt, 1982).

2.8. N PROCESSES IN SOIL

2.8.1. N_2 fixation

Although there is a vast supply of N in the Earth's atmosphere, it cannot be used by higher forms of plants and animal life. The only form of life that can use it is free-living (non-symbiotic) soil bacteria, blue-green algae (cyanobacteria) and symbiotic bacteria (e.g. *Rhizobium*) in the root nodules of legumes and certain other plants (Havelka et al., 1982; Stevenson, 1982; Campbell et al., 1999). N_2 is converted into an inorganic form of N such as NH_3 (equation 2), which can be used to synthesize nitrogenous organic compounds, such as amino acids, proteins, vitamins and nucleic acids (equation 3). N_2 fixation is a very important process in the build-up of the available nitrogen pool, but it only contributes a tiny fraction of the nitrogen that is annually assimilated by agricultural crops (Campbell et al., 1999) although in natural ecosystems N_2 fixation is very important.

$$N_2 + 8H^+ + 6\ e^- \rightarrow 2NH_3 + H_2 \qquad \text{(eq.2)}$$

$$NH_3 + \text{organic acids} \rightarrow \text{amino acids} \rightarrow \text{protein} \qquad \text{(eq.3)}$$

2.8.2. Nitrification

Nitrification is performed by bacteria that live in the soil. Factors such as organic matter, water content, oxygen supply, temperature and soil pH can affect how much, or how little, ammonium (NH_4^+) is converted to nitrate (NO_3^-). Warm, moist soils with good oxygen supply provide favourable conditions for nitrification. Nitrification is very active during the spring and summer months, but declines in the autumn and is essentially non-existent during the winter (Muextension, 2005).

There are two stages in the nitrification process, first the oxidation of NH_4^+ to NO_2^-, and then the oxidation of NO_2^- to NO_3^-. The second reaction is more rapid. Thus, in natural soils the fraction of NO_2^- is small. The nitrification process is primarily accomplished by two groups of autotrophic nitrifying bacteria.

In the first step of nitrification, the ammonia oxidizing bacteria, *Nitrosomonas*, oxidize ammonia to nitrite according to equation (4).

$$NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^- \qquad \text{(eq.4)}$$

In the second step of the process, nitrite oxidizing bacteria, *Nitrobacter*, oxidize nitrite to nitrate according to equation (5) (Stevenson, 1982; US EPA, 2005a).

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^- \qquad \text{(eq.5)}$$

Nitrate released from these bacteria can then be assimilated by plants and converted to organic forms, such as amino acids and proteins (Campbell et al., 1999). One result of this oxidation reaction is that the pH of the soil decreases, which causes acidification (Van Niekerk, 2004).

2.8.3. Denitrification

This process is conducted by facultative anaerobe bacteria that are distributed in soils, sediments and water (Stevenson, 1982). Most of these bacteria are heterotrophic (e.g. *Pseudomonas*, *Bacillus* and *Micrococcus*) and get their energy from carbon and organic compounds, but they can also be autotrophic (e.g. *Thiobacillus*) and get their energy from sulphide compounds. They use NO_3^- as an oxidizing agent in an environment depleted of oxygen. The result is the reduction of NO_3^- to N_2 and N_2O and these two gases eventually become part of the atmosphere:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \text{(eq.6)}$$

Since denitrification is strongly dependent on organic compounds, the addition of organic materials such as plant residues or manure greatly increases the denitrification activity (Firestone, 1982; Stevenson, 1982; Ingesson, 1996). The rate of denitrification is much lower in acid soils. The amount of N_2 returning to the atmosphere is relatively small.

2.8.4. Mineralization and Immobilization

Mineralization is the process by which soil organic N is transformed to inorganic NH_4^+ with the help of bacteria and fungal decomposers. This process recycles large amounts of nitrogen back to the soil and therefore plays a key role in the N cycle. These heterotrophic organisms derive energy from the oxidation of organic compounds either by consumption or absorption of other organisms (Campbell et al., 1999). Immobilization works in the opposite way, when heterotrophic microorganisms utilize inorganic N compounds (NH_4^+, NH_3, NO_3^- and NO_2^-) and transform them into organic constituents of their tissues and cells (Jansson & Persson, 1982; Stevenson, 1982).

$R - NH_2 \leftrightarrow OH^- + R\text{-}OH + NH_4^+$ (eq. 7)

Mineralization depends on climatic factors such as temperature, moisture and also pH in the soil, which affect the microbial activity (Leiros et al., 1999).

Temperature

Arrhenius (1889) discovered that chemical reactions proceed faster at high temperatures. Mineralization of nitrogen has been shown to continue to an upper temperature of 35°C, but in nature the activity is normally reduced at these high temperatures (Harmsen & Kolenbrander, 1965). According to Grundman et al. (1995), the temperature for maximum mineralization activity may depend on the temperature regime of the soil, as the microorganisms adapt to the temperature of their soil habitats. At temperatures below 0°C, the microbial activity is low and restricted to the availability of unfrozen water, which occurs in the smallest micro-pores. These pores can keep water in liquid form at low matric potentials. Indeed certain bacteria are adapted to cold environments, e.g. tundra climate, and some have the ability to decompose organic material down to -7°C (Flanagan & Veum 1974 cit. Wennman, 2004).

Soil water

Microbial activity is dependent on soil water and increases up to optimal moisture levels at 0.1-0.3 bar (field capacity) (Goncalves & Carlyle, 1994; Leiros et al., 1999). At water contents below wilting point (15 bar) or above field capacity, the activity decreases rapidly (Grundman et al., 1995; Wennman, 2004).

pH

The pH is very important for the microbial activity of the soil and the N mineralization is affected positively if pH is increased as a result of liming treatment. It is also known that mineralization occurs over a broad range of pH levels without a marked reduction in activity (Page, 1974; Korentaljer, 1991; WRC, 1997; Wennman, 2004). Soils should have a pH of 6.5 or above. A pH drop could cause an increased mobility of heavy metals, which may affect the organism community.

2.8.5. Ammonia Volatilization

Volatilization of ammonia can occur whenever free NH_3 is available near the soil surface. An increase in soil pH and temperature increases the loss of ammonia (eq. 8) (Stevenson, 1982a).

$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O$ (eq.8)
Ammonia (gas)

2.8.6. Leaching

Leaching is the loss of soluble NO_3^- as it moves with soil water, generally excess water, below the root zone. Nitrate that moves below the root zone has the potential to enter either groundwater or surface water through drainage systems (Extension, 2005). The main loss of N by leaching is through NO_3^- when precipitation exceeds evapotranspiration.

Coarse-textured soils have a lower water-holding capacity and therefore a higher potential to lose nitrate through leaching compared with fine-textured soils. The movement of NO_3^- is closely related to water movement (Extension, 2005). Leaching is strongly influenced by seasonal effects such as water and temperature. In humid temperate regions, mineralization rates are low in winter, but leaching of residual NO_3^- from the previous season often occurs. In the spring, NO_3^- accumulates as nitrification rates increase and N fertilizer is applied. If heavy rains occur before planted crops are growing strongly at the start of the growing season, important amounts of NO_3^- can be lost below the root zone. Nitrate leaching is lowest during the summer, because evapotranspiration usually exceeds precipitation and plant uptake rates are high (Legg & Meisinger, 1982).

2.8.7. Erosion and runoff

Most of the N that is lost by soil erosion is in organic forms and does not represent a loss of available N but there is a large loss of nutrients such as nitrate and ammonium in runoff from surface waters. Ammonium ions attach to sediments very readily, which means they move with soil, but generally do not leach. Therefore, ammonium may contribute to surface water problems, but generally does not impact on groundwater. Losses through these events do not normally account for a large proportion of the soil N budget, but should be considered for surface water quality issues (Legg & Meisinger, 1982; Extension, 2005; Ohio State, 2005).

3. METHODOLOGY

3.1. EXPERIMENTAL DESIGN

In the present study, 300 samples were batch-incubated for 28 days at 25°C. A sub-sample of 180 of these were analyzed for N and 120 for pH. There were 60 new samples incubated each week, 20 samples from each treatment. The treatments were sludge (Sludge), ammonium nitrate (AN) and control (C) and the samples were adjusted to four different water potentials, 1, 5, 10 and 13.8 bar. Five replicates were taken for each treatment and three of these were analyzed for inorganic N and the remaining two for pH and water potential. Samples were taken on days 1, 7, 14, 21 and 28.

3.2. SUBSTRATE PROPERTIES

Soil

The soil used was collected from the Hatfield Experimental Farm, University of Pretoria, South Africa (28º16′E, 25º45′S) at an altitude of 1327 metres above sea level. According to particle analysis, the soil is classified as a sandy clay loam (Table 5) (Brady & Weil, 1999). Table 6 shows some selected chemical properties of the soil. The climate in the region can be described as semi-arid with an average annual precipitation of 670 mm, most of which falls during the rainy season from October to

March (Tesfamariam, 2004). The monthly average maximum temperature is 30ºC (January), with a monthly average minimum of 1.5ºC (July). Frost occurs during winter.

Table 5 *Particle size analysis of the soil*

Sand (%)	Silt (%)	Clay (%)	Total (%)
72.3	6.7	21.0	99.9

Table 6 *Chemical properties of the soil*

pH (H_2O)	P mg kg^{-1}	Ca mg kg^{-1}	K mg kg^{-1}	Mg mg kg^{-1}	Na mg kg^{-1}	C %
5.9	3.2	232	24	92	16	0.18

Sludge

The sludge was collected at the Olifantsfontein Waste Water Treatment Plant. The sludge was first air-dried at the Hatfield Experimental Farm in a greenhouse and later pulverized at the Agriculture Research Council, Institute of Soil Climate Water laboratory. The sludge was both air-dried and oven-dried at 56°C (Table 7) prior to analysis for total N and C.

Table 7 *Total C and N content (%) of air-dried and oven-dried sludge*

Sludge		**Ave**	**Std**	**CV (%)**	**C:N**
Air-dried	**N**	5.12	0.036	0.703	
	C	35.04	0.025	0.071	6.8
Oven-dried 56°C	**N**	5.34	0.015	0.281	
	C	36.91	0.275	0.766	6.9

Ammonium nitrate

A relatively pure salt of ammonium nitrate (AN) was obtained from the laboratory at the University of Pretoria. AN contains 35.15% N calculated from the periodic table.

3.3. PREPARATION

3.3.1. Preparation of samples

In South Africa, the restriction on sludge utilization in agriculture is set at 8 tonnes dry matter ha^{-1} year^{-1}. This amount was thus used in this incubation study. To calculate the equivalent amount of sludge that must be used in the batch experiment the amount of sludge must be converted from mass of sludge per area to mass of sludge per mass of soil. In order to do this, a soil depth of 30 cm and a bulk density of 1.289 g cm^{-3} were chosen. The exact amount of bio-available N from sludge was not known and therefore both treatments started with the same amount of N. This corresponded to 0.111g dry sludge and 0.0056832 g of N per 50 g soil.

The bulk density chosen falls inside the typical bulk density range for disturbed cultivated topsoils.

An equivalent amount of mineral N was then added to the sample in AN (for calculations, see Appendix 1).

3.3.2. Preparation of water potentials

Before the different water potentials could be established, the relationship between water content (Θ_g) and matric potential (Ψ_m) (the water retention characteristics) of the soil had to be established. Water retention characteristics of a soil are a function of both texture and bulk density (ρ_b), and therefore the water retention characteristics of a soil must be determined at a known and constant ρ_b.

A ceramic pressure plate apparatus was used to determine the relationship between (Ψ_m) and (Θ_g) of the soil. The following procedure was used to ensure that the ρ_b of the samples were the same.

Steel rings was packed to the same volume and amount of soil. The same packing procedure was followed meticulously. A total of 87g of soil or soil/sludge mixture was packed in the rings, which were 3 cm thick and occupied a volume of 67.49cm^3 ($V_T = \pi * r^2 * h = \pi * 2{,}676^2 cm * 3cm$).

The ρ_b was calculated to $\rho_b = m_s/V_t$, 87/67.49=1.289 g cm^{-3}. The rings were placed on ceramic plates with different suctions. The rings with the soil as well as the ceramic suction plates were saturated for 24 hours with deionised water. Each treatment was replicated three times. After saturation the ceramic plates were placed in the pressure plate apparatus at the chosen suction for 2-3 days. Once equilibrium was achieved, the suction in the chamber was equalized to the matric potential of the remaining water in the soil sample. The samples were removed and weighed to determine the amount of water remaining. The amount of water that was left in the soil was equivalent to the soil water content at that given potential. The samples were weighed, oven-dried at 105ºC for 48 hours and re-weighed. The water retention curve shows the relationship between the water content in the soil and the matric potential.

3.3.3. Preparation of water measurements

Before the pre-incubation started, three replicates from each potential were weighed. During half an hour of aeration the bottles were allow to evaporate water and then weighed again. The weight loss was assumed to be only a loss of water. This amount of water was added throughout the whole incubation after half an hour of aeration.

During the whole incubation, the bottles were opened for half an hour every second day and at the end of each week water was added to bring back each sample to the chosen water potential. Water was assumed to only be lost during this half hour of aeration.

3.4. INCUBATION PROCEDURE

3.4.1. Pre-incubation of soil

The soil samples were pre-incubated at a constant temperature of 25ºC at the four different water potentials for one week. The reason for the pre-incubation was to ensure that the microbial population adapted to experimental conditions.

The soil was aerated every second day for half an hour and deionised water was added once a week to bring back the soil to the chosen water potential.

3.4.2. Incubation

After the seven days of pre-incubation, sludge and ammonium nitrate were added according to the different treatments. The samples were well mixed and packed at the bulk density of 1.289 g cm^{-3}. Every second day the samples were aerated and once a week they were brought back to the chosen water potential as for the pre-incubation trial.

3.5. N ANALYSES

Inorganic N was determined with the Kjeldahl digestion method.

After the batch incubation was finished, 100ml of 1M KCL were added to each container that contained 50 g of soil. The samples were mixed and shaken for one hour and the KCL extract was distilled in order to determine the inorganic N in the extract. A 50 ml aliquot of the extract was used for the inorganic N analysis. 20 ml of a 50% NaOH was added to the aliquot as well as a spatula of Devarda´s alloy.

During the Kjeldahl digestion process, all NO_3^--N in the solution was reduced to NH_4^+-N by Devarda´s alloy. Then all NH_4^+-N volatilized as NH_3 and condensed.

$$NH_4^+ \Rightarrow NH_{3\,(g)} \Rightarrow NH_{3\,(ag)} \qquad \text{(eq.9)}$$

$$\underset{\text{(Green)}}{NH_{3\,(ag)} + H^+} \Rightarrow NH_4^+\,BO_3 \qquad \text{(eq.10)}$$

All the NH_4^+-N was then collected in a new bottle containing boric acid (H^+). The solution changed colour from purple to green. The reduction of NH_4^+-N continued until there were about 250 ml of solution. This green boric acid solution was then titrated with 0.01M HCl until a colour change back to purple occurred.

$$\underset{\text{Green}}{[NH_4^+\,BO_3]} + 3H^+ \Rightarrow \underset{\text{purple}}{[H_3\,BO_3]} + NH_4^+ \qquad \text{(eq.11)}$$

The volume of added HCl was used to calculate the total NH_4^+-N in the incubated soil (for calculations see Appendix 2). 50 ml filtered 1 M KCL were used as a blank sample and subjected to the same procedure as the rest of the samples.

3.6. DETERMINATION OF pH

The pH was determined in all samples. 125 ml of H_2O was added to each soil sample of 50g (ratio 2 to 5). The sample was mixed with a glass rod and then pH was measured after 30 minutes.

3.7. WATER POTENTIAL DETERMINATION

To see whether the water potentials stayed constant throughout the whole experiment, water potential measurements were carried out after the incubation was finished using a Dew Point Potentiameter, (model WP4) (Decagon Devices, Inc., Pullman, WA[1]) (water activity meter). The readings were taken in MPa. WP4 was first calibrated by measuring the water potential of a salt solution with a known osmotic potential. This incubation study used a 0.5 M KCl solution that had a value of -2.22 MPa. The WP4 measures the water potential of a sample by determining the relative humidity of the air above the sample in a closed chamber. At temperature equilibrium, relative humidity is a direct measurement of water potential, so after measuring a sample, the instrument converts the readings to water potential units (MPa).

A temperature check is important because if the laboratory and WP4 temperatures fluctuate by ± 5°C daily, water potential readings can vary as much as ± 0.5 MPa on a dry soil sample.

The measurements took approximately 5 minutes and if the temperature difference was large the readings could take up to 15 minutes (Scanlon et al., 2002).

3.8. STATISTICS

To see whether the differences between the three treatments were statistically significance, an analysis of variance (ANOVA table) was done. In cases where significance was shown, a t-test was done to test this at a 95% confidence level ($p < 0.05$). The ANOVA table was prepared using the statistical programme Genstat (General Statistics developed by VSN International Ltd). The statistical analysis was made on differences in inorganic N and pH. For differences between treatments in pH, LSD values were used for measuring the significance. No statistical analysis was made of the water potential measurements since there were only one or two samples of each treatment.

4. RESULTS

4.1. WATER RETENTION CURVE

Before the incubation started, water retention characteristics for C and Sludge were determined (Fig 2). The water retention curve shows the relationship between the water content in the soil and water potential.

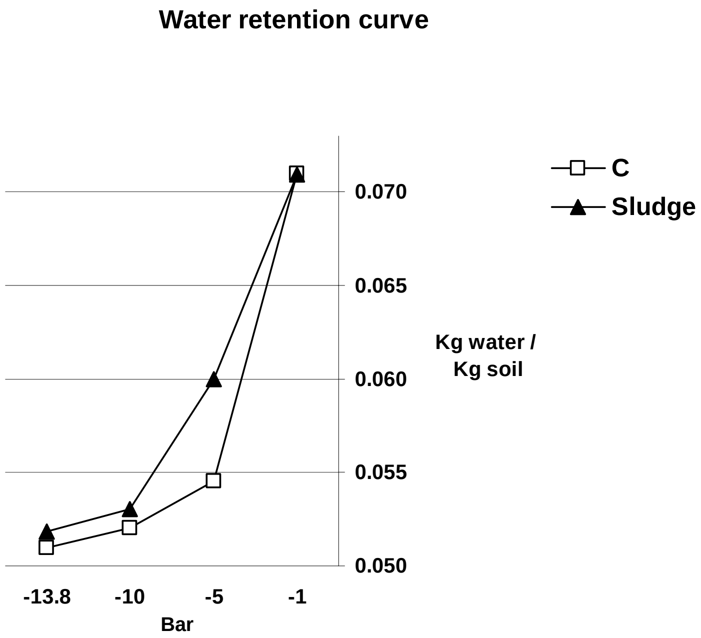

Figure 2 *Soil water content as a function of water potential (Water Retention Curve) for Sludge and C.*

Figure 2 shows that sludge-amended soil contains more water under dry conditions than C. The addition of organic material increases the amount of mesopores and thereby the ability to hold more water when the soil becomes drier.

The water retention curve for C was the one used for all three treatments during the incubation.

4.2. WATER POTENTIAL MEASUREMENTS

The water content could not be kept constant during the whole incubation. More water evaporated from the bottles than was added each week and this resulted in very dry soil at the end of the incubation. Water was lost from the bottles even when the lids were closed. The amount of water that left each bottle was unfortunately not determined. Therefore, the effect of the different water potentials on N mineralization that I intended to quantify could not be evaluated statistically.

To quantify how much water was lost, water potential measurements were conducted during the incubation with a WP4 (see Section 3.7). WP4 showed that potentials were about 6 times lower than intended at the beginning and as much as 60 times lower at the end of the incubation. This means that WP4 did not give accurate results. For exact values see Appendix 5.

4.3. INORGANIC N

4.3.1. Control

The dynamics of mineral N showed no clear differences between the four water potentials (Fig. 3). According to analysis of variance the effect of time was significant (Pr 0.026). Testing differences between sampling dates (t-test) revealed significant differences only between day 1 and 28 for 1 and 13.8 bar. On day 28, inorganic N was significantly higher than on day 1. Not surprisingly, the amount of mineral N increased with time. At 5 and 10 bar, time had no effect on the amount of mineral N. More detailed inorganic N values and statistics data are given in Appendix 3a and 6a.

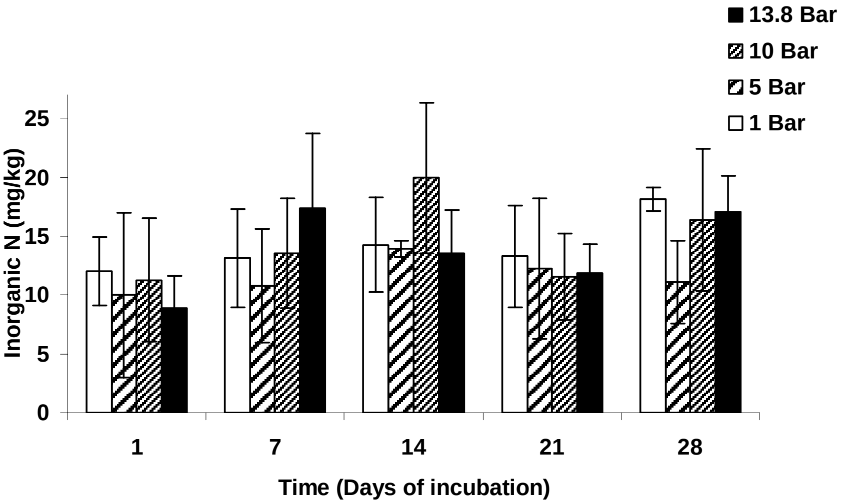

Figure 3 *Inorganic N in mg kg^{-1} for C at 1, 5, 10 and 13.8 bar on the different sampling dates. The values are mean values and the error bars represent their standard deviation.*

4.3.2. Sludge

In Sludge, mineral N increased highly significantly ($p<0.001$) with time (Fig. 4). Here, the effect of water potential on mineral N was also significant ($p<0.002$). However, since the water potential could not be controlled satisfactorily during the incubation, no quantitative conclusions can be drawn from this experiment (see Section 4.2). According to t-tests, the increase in mineral N with time was highest at 1 bar, i.e. mineral N was significantly lower on days 1 and 7 than on days 21 and 28. The increase in mineral N with time was less pronounced towards the end of the incubation at the higher potentials. At 5 bar, differences were significant between day 28 and day 7, whereas changes in mineral N in time were not significant at 10 and 13.8 bar. More detailed inorganic N values and statistics data are given in Appendix 3b and 6b.

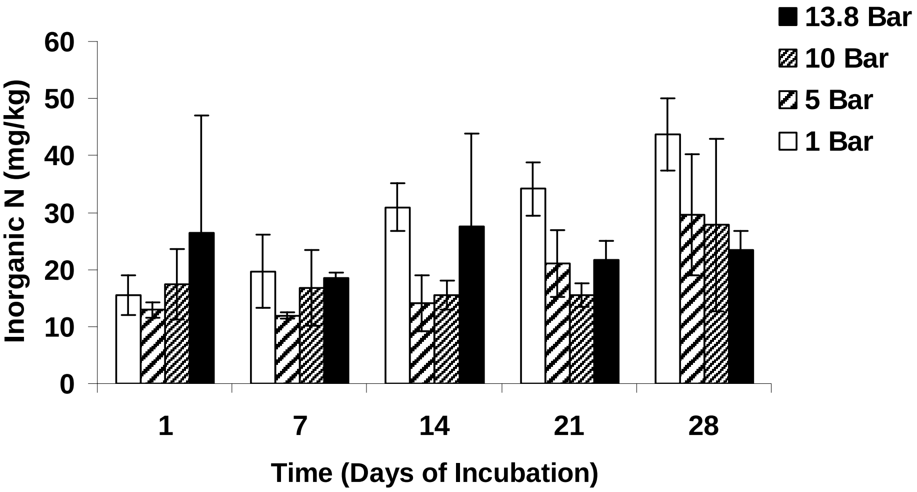

Figure 4 *Inorganic N in mg kg^{-1} for Sludge at 1, 5, 10 and 13.8 bar on the different sampling dates. The values are mean values and the error bars represent their standard deviation.*

4.3.3. Ammonium nitrate

The amounts of mineral N in AN were at least 10 times higher than those in C and Sludge (Fig. 5).

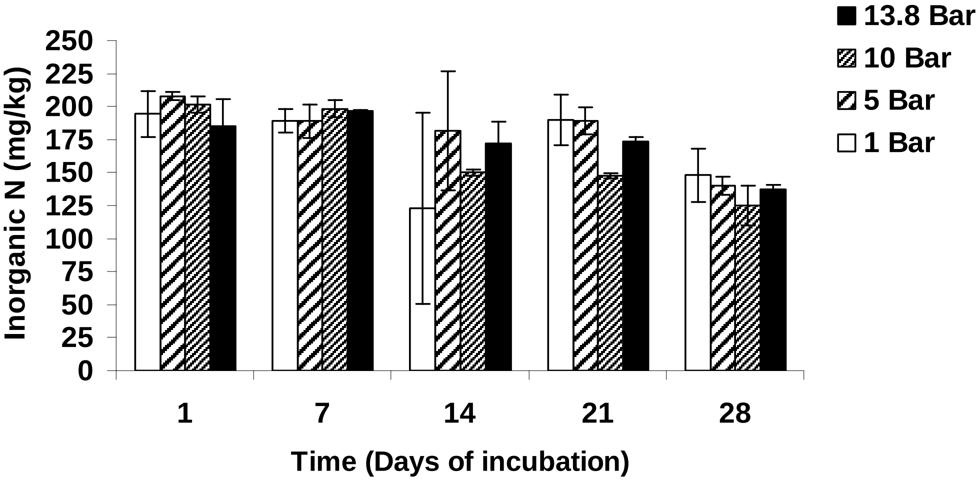

Figure 5 *Inorganic N in mg kg^{-1} for AN at 1, 5, 10 and 13.8 bar on the different sampling dates. The values are mean values and the error bars represent their standard deviation.*

In this treatment too, the effect of mineral N was highly significant (p<0.001). However, here the amount of mineral N decreased with time.

This decrease was most pronounced for 5 and 10 bar, where inorganic N was significantly lower at day 28 than at days 1 and 7. At 1 and 13.8 bar, this decrease was significant between days 7 and 28. More detailed inorganic N values and statistics data are given in Appendix 3c and 6c.

4.3.4. Comparison of treatments

The amount of inorganic N in Sludge at 1 bar was significantly higher on days 14, 21 and 28 than that in C. The AN at 1 bar on days 28, 21, 7 and 1 also showed significantly higher N amounts than both C and Sludge.

This means that mineral N was higher in AN than in the other treatments during almost the whole experiment and higher in Sludge than in C during the second half of the experiment.

4.4. pH

4.4.1. Control

According to analysis of variance, neither time nor water potential had any significant effect on the pH in C. The pH for all four water potentials was between 5.34 and 5.88 (Fig. 6). For more details see Appendix 4a and 7a.

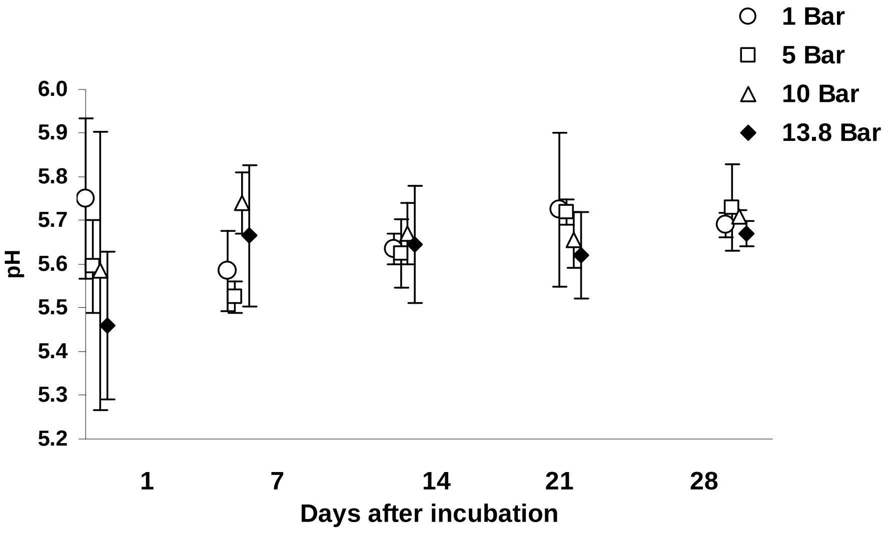

Figure 6 *pH for C at 1, 5, 10 and 13.8 bar on the different sampling dates. The values are mean values and the error bars represent their standard deviation.*

4.4.2. Sludge

The dynamics of pH in Sludge were similar to those in C. Neither time nor water potential influenced the pH significantly (Fig. 7). The pH for all four water potentials was between 5.22 and 5.95. For more details see Appendix 4b and 7b.

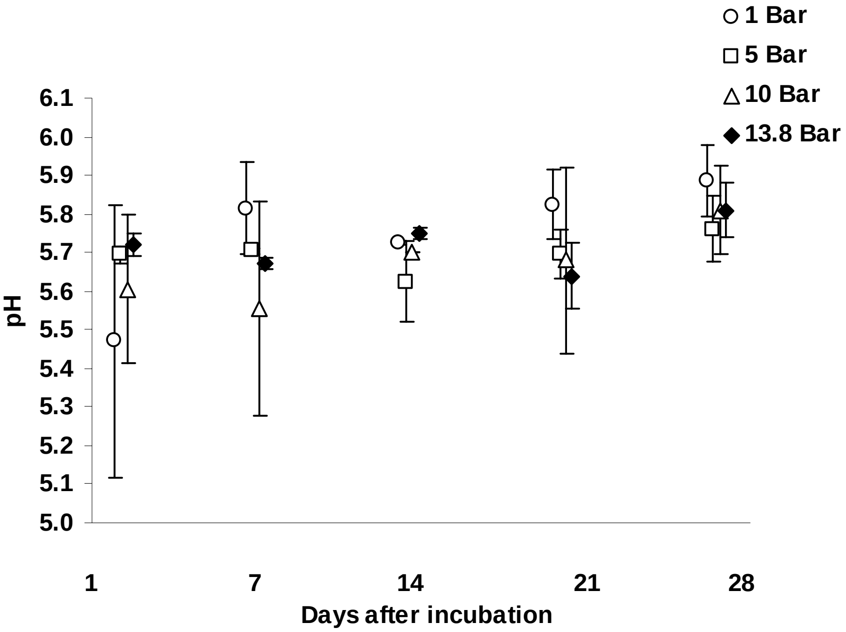

Figure 7 *pH for the Sludge treatment at 1, 5, 10 and 13.8 bar on the different sampling dates. The values are mean values and the error bars represent their standard deviation.*

4.4.3. Ammonium nitrate

The effect of time on mineral N was highly significant ($p<0.001$). The pH decreased and the soil became more and more acidified with time (Fig. 8). The decrease in pH was probably due to the oxidation of NH_4^+ to NO_3^-. This process generates H^+ ions, which acidify the soil. As for C and Sludge, the different water potentials did not have an effect. The pH for all four water potentials was between 4.83 and 5.39. For more details see Appendix 4c and 7c.

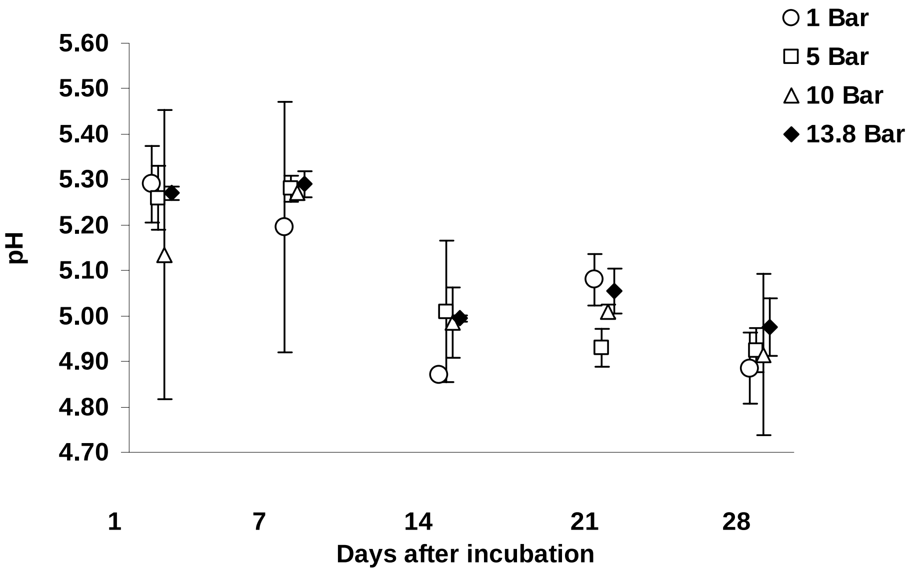

Figure 8 *pH for AN at 1, 5, 10 and 13.8 bar at the different sampling dates. The values are mean values and the error bars represent their standard deviation.*

4.4.4. Comparison of treatments

According to analysis of variance, AN had significantly lower pH compared with both C and Sludge (Table 8). This was the case irrespective of both water potential and days after incubation day (Appendix 7d).

Table 8 *Mean pH values in C, Sludge and AN. Different letters indicate significant differences between treatments. LSD stands for least significant differences between means (p<0.05)*

	Treatments		
	Control	**Sludge**	**Ammonium nitrate**
Mean value	5.65a	5.71a	5.08b
LSD	0.056		

5. DISCUSSION

5.1. PARTICLE SIZE ANALYSIS

On the basis of its texture, the soil used here is classified as a sandy clay loam, with 72.3% sand, 6.7% silt and 21.0% clay. According to NMSU (2005), sandy clay loams contain usually 20% water at field capacity and have a dry bulk density of about 1.5 g cm^{-3}. From the water retention curve (Fig. 2) we can see that the water content for the soil used in this incubation is very narrow and varies only between 5 and 7% for all four water potentials applied in this study. One reason for this might be that the pressure plate apparatus did not work properly. What we can see is that only 2% water disappeared from the soil as the soil potential became 10 times more negative. The other reason might be that since the water retention curve is determined by the pore size distribution, which in turn is a function of texture, structure and bulk density of the sample, the water retention curve can look very different in disturbed samples. That means that the same soil packed at different bulk densities will result in different water retention characteristics. Another possible explanation is therefore that the soil was compacted more than intended, which resulted in a decrease in micro- and mesopores.

5.2. WATER POTENTIAL MEASUREMENTS

One of the objectives of this experiment was to examine the influence of different water potentials on the (N) mineralization in the soil.

The reason for the specific experimental conditions chosen in this experiment was because of the geographical situation in which the experiment was conducted, namely South Africa. Under a field situation, sludge would have been incorporated into the top 30 cm of the soil, which is the part of the soil most exposed to extreme moisture conditions. These can vary from virtual desiccation in the dry season to very wet conditions during heavy rain.

The geographical situation was also the reason for choosing the four water potentials (1, 5, 10 and 13.8 bar). In the field, the wilting point for plants is at 15 bar, while the optimal moisture level in the soil is at field capacity (0.1-0.3 bar). The reason for not choosing the potential for optimal moisture level in the soil is that we wanted to focus on the N mineralization on the driest zone of the soil.

One of the major controllers of the microbial activity in the soil is the water content. Generally microbial activity increases with water content between wilting point and field capacity. Above field capacity, the activity decreases due to lack of oxygen (Goncalves & Carlyle, 1994; Leiros et al, 1999).

Unfortunately, the water potentials could not be controlled properly in this incubation experiment. Although the effect of the different potentials on the N mineralization was significant in some cases, the results are only of a qualitative nature. The increasing water loss with time that was visually obvious was confirmed by measurement using a Dew Point Potentiometer (model WP4) at the end of the experiment, when the soil was very dry. I adjusted the water content for the time when the bottles were aerated but did not realise that water was lost all the time, since the lids did not seal properly.

Bottles with airtight lids should be used in further investigations. An easier way of measuring water loss from the vessels would have been to dry some of the samples and to determine water loss gravimetrically.

5.3. INORGANIC N

The statistics show that AN had significantly higher inorganic N values during the whole incubation period than both the C and Sludge treatments. In AN the amount of mineral N decreased with time while it increased in the other two treatments. Mineral N in Sludge was significantly higher than in C. For all three treatments, time influenced the amount of mineral N significantly ($p<0.05$).

One of the questions in the beginning concerned the size of the bio-available N fraction in sludge. If that had been an exact and constant amount, the equivalent amount of inorganic N would have been added to AN. The bio-availability of N was not known and it was therefore decided that both Sludge and AN should start with the same amount of total N. However the inorganic N amounts of the two treatments differed significantly.

At the beginning of the incubation, the nitrogen in AN was already in an inorganic N form while the nitrogen in Sludge had to be mineralized. Thus the assumption made in the beginning was false since not all N in sludge was bio-available as in AN. This can also be seen comparing Figures 4 and 5, where the initial inorganic N values are not the same for the two treatments. Since we did not know before the incubation started how large the inorganic N fraction in Sludge was, we overestimated rather than underestimated the amount of inorganic N in sludge. If further incubation is carried out, proper estimations of the mineralization of inorganic N in sludge have to be made before and after a longer time, e.g. several weeks to months. To assume that 100% N in sludge is bio-available from the beginning is not correct.

For both C and Sludge, the mineralization increased with time as the microorganisms broke down the organic matter in the soil and mineral N was released. This process continues in the soil as long as conditions are not too dry or too wet. The reason that Sludge contained higher amounts of inorganic N was because extra organic material was added to the soil in that treatment, so the microorganisms had more organic matter to mineralize. According to a study done by Jansson & Persson (1982) and Goncalves and Carlyle (1994), the mineralization of organic N (and C) in the soil is stimulated largely by the quantity and quality of organic matter but it is also influenced by environmental factors, principally moisture and temperature.

This trend was also expected for the AN, but unexpectedly the opposite occurred and mineral N decreased with time. This reduction in inorganic N might be due to the environment in the incubation bottles being favourable for different kinds of N losses. Leakage of N can be excluded, since the incubation bottles were closed at the bottom. Thus, denitrification of soil N might be a possible process accounting for the N losses. Two ways that N might have disappeared are through dinitrogen gas (N_2) and dinitrogen oxide gas (N_2O). Denitrification in the soil is favoured by many factors such as low oxygen content, high organic matter content, availability of N as NO_3^- and temperature in the soil. In this incubation study the temperature was constant at 25°C, so the soil was relatively warm all the time, and there was a constant supply of available NO_3^-. According to Williamson & Johnson (1994), denitrification is promoted by an increase in soil temperature and the main product is dinitrogen gas, but with time the production of nitrogen oxide (NO) increases in the soil. In their incubation during day 21, NO gas represented 99% of the total N gas production in the soil (Williamson & Johnson, 1994). However, one factor that might have limited the N_2 production in the present incubation was the low soil moisture status in the soil. Due to the dryness of the soil at the end of the incubation, the material became very compacted and that may have contributed to reduced oxygen content in the soil, which can lead to a several fold increase in N_2O production in the soil. This was the case for Groenestein & van Faassen (1996).

A low pH may also limit the denitrification in some soils but this was probably not the case here since the pH was over 5 and denitrification is negligible at $pH < 4$ (Brady & Weil, 1999).

Another way for N to disappear from the soil might have been through volatilization of ammonia. From the literature we know that ammonia is formed when organic material breaks down and the gas is easily dispersed. Ammonia is about 60% lighter than air. According to Williamson & Johnson (1994) and Zucong et al. (2001), loss of nitrogen as volatile ammonia is relatively minor in soil with a $pH < 7$ and it is only at a $pH > 9$ that ammonia gas increases in the soil. So according to those findings, volatilization of ammonia was probably not a great problem in the present study. Another explanation for this decrease in inorganic N may be that the conditions in the soil became more favourable for the microorganisms, so they started to immobilise the N as organic N in their tissues and cells. Unfortunately this may not be the answer either, since the soil was very dry and microorganisms rarely grow under these conditions.

It is apparent from the data presented that the most significant results for all three treatments occurred at the lowest water potential, while at more negative water potentials there was less difference in mineralization between days. The amount of water is very important for the mineralization of nitrogen and there is a lot of activity in the soil at field capacity (0.1-0.3 bar). The lowest water potential that I chose in this incubation was already 10 times drier than field capacity and therefore I would recommend students in further research in this area to use field capacity. Goncalves & Carlyle (1994) and Leiros et al. (1999) found that mineral nitrogen production increased linearly with time for most combinations of moisture and temperature. Furthermore, the rates of production showed no tendency to decrease with time, except at low soil moisture. Those researchers found that N was still mineralized after 60 days of incubation.

5.4. pH

The second aim was to test how pH changed with time during the incubation at different water potentials. When all three treatments were compared together, the t-test showed that AN had a significantly lower pH compared with the other two treatments (Table 8). Furthermore, the t-test on the results from the pH measurements showed no influence of time in either the C and the Sludge treatments but a high significance of time ($p < 0.001$) for the AN treatment.

This shows that only the AN treatment had an effect on soil pH. With time, NH_4^+ was oxidized to NO_3^- and released H^+ ions and the pH decreased as the soil became more and more acidified. This acidifying effect may have a negative influence on the microbial flora in the soil and therefore the mineralization process. Another negative effect that may happen in an acid soil is that the mobility of specific metals may increase, which in turn may harm the soil flora growing there. A decrease in pH may also lead to farmers having to lime the soil to maintain favourable conditions for both microorganisms and plant roots. Acid soils affect plant growth by creating toxicities and nutrient deficiencies, reducing plant root growth and decreasing legume nodulation.

However, this is not a great problem in soils with a pH around 5 but it would be a problem if the pH decreased more. If that were to happen, I would recommend that farmers do not use ammonium nitrate as a fertilizer in agriculture, but to use sewage sludge instead! Of course this is not something I can really speak about, since further studies including plants would be needed first.

6. CONCLUSIONS

In this incubation study some differences were found between the three treatments.

- For both C and Sludge, mineralization increased significantly with time, as the organic N produced by microbial activity was mineralized to inorganic N.
- For AN this mineralization was negative and might have been due to loss of N through N_2, N_2O and NO. However, this decrease was very unexpected.
- The pH for the AN decreased significantly with the N mineralization, due to oxidation of ammonium to nitrate.
- The pH for the other two treatments increased with time, but this increase was not significant.

Due to these results I would recommend usage of sludge as a fertilizer in agriculture but before that, more research has to be done in which the influence of plants is examined. One important aspect in further research is that a more accurate incubation method has to be used and evaluated.

7. REFERENCES

Published sources:

Arrhenius, S. 1889. Über die Reaktionsgeschwindigkeit bei der Inversion von Rohrzucker durch Säuren. *Zeitschrift för Physik Chemique* 4: 226-248.

Borain, G.P. 1993. The use of sewage sludge in agricultural economics. *In* Ekama, G.A. (ed): *Sewage Sludge Utilization and Disposal*. Water Institute of Southern Africa.

Brady, N.C. & Weil, R.R. 1999. *Nature and Properties of Soils*. 12th edition., Prentice Hall, Inc. Upper Saddle River.

Bremner, J.M. 1965. Inorganic forms of nitrogen. *In* Black, C.A., Evans .D.D., White, J.L., Ensminger, L.L. & Clark, F.E. (eds): *Methods of Soil Analysis*. American Society of Agronomy, Madison, pp. 1179-1237.

Campbell, N.A., Reece, J.B. & Mitchell, L.G. 1999. *Biology*. 5th edition.

Chaney, R.L., Bruins, R.J.F., Baker, D.E., Korcak, R.F., Smith, J.E. & Cole, D. 1987. Transfer of sludge-applied trace elements to the food chain, Chapter 5. *In* Page, A.L., Logan, T.J., Ryan, J.A. (eds): *Land Application of Sludge-Food Implications*. Lewis publisher Inc., USA.

Corey, R.B., King, L.D., Lue Hing, C., Fanning, D.S., Street, J.J. & Walker, J.M. 1987. Effects of sludge properties on accumulation of trace elements by crops, Chapter 3. *In* Page, A.L., Logan, T.J., Ryan, J.A. (ed): *Land Application of Sludge-Food Implications* (ed). Lewis publisher Inc. USA.

Dahlin, S., Kirchmann, H., Kätterer, T., Gunnarsson, S. & Bergström, L. 2005. Possibilities for improving nitrogen use from organic materials in agricultural cropping systems. *Ambio* 34: 288–295.

Davis, R.D. 1987. Use of sewage sludge on land in the United Kingdom. *Wat.Sci.Tech* 19: 1-8.

Davis, R.D. 1992. Discussion on Europe's mountainous problem. *Water Quality International* 3: 22.

Department of National Health and Population Development. 1991. *Permissible Utilization and Disposal of Sewage Sludge,* A11/2/5/4, December 1991. Private Bag X828, Pretoria, 0001.

Ekama, G.A., 1993a. Sludge management for land disposal. *In* Ekama, G.A. (ed): *Sewage Sludge Utilization and Disposal*. Water Institute of Southern Africa.

Falcone, A.B., Shug, A.L. & Nicholas, D.J.D. 1963. Some properties of a hydroxylamine oxidase from *Nitrosomonas europaea*. Biochim. Biophys. Acta 77: 199-208.

Firestone, M.K. 1982. Biological denitrification. *In* Stevenson, F.J. (ed): *Nitrogen in Agricultural Soils*, American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc Publisher Madison, Wisconsin USA. Agronomy 22: 289-326.

Goncalves, J.L.M. & Carlyle, J.C. 1994. Modelling the influence of moisture and temperature on net nitrogen mineralization in a forested sandy soil. *Soil Biology and Biochemistry* 26: 1557-1564.

Groenstein, C.M. & Van Faassen, H.G. 1996. Volatilization of ammonia, nitrous oxide and nitric oxide in deep-litter systems for fattening pigs. *Journal of Agricultural Engineering Research* 65: 269-274.

Grundmann, G.L., Renault, P., Rossa, L., Bardin, R., 1995. Differential effects of soil water content and temperature on nitrification and aeration. *Soil Science Society of American Journal*:59, 1342-1249.

Hansen, E.M. & Djurhuus, J. 1997. Nitrate leaching as influenced by soil tillage and catch crop. *Soil & Tillage Research* 41: 203-219.

Harmsen, G.W. & Kolenbrander, G.J. 1965. Soil inorganic nitrogen. *In* Bartholomew, W.V. & Clark, F.E. (eds): *Soil Nitrogen, No 10*, American Society of Agronomy, Madison, Wisconsin. pp. 43-

Havelka, U.D., Boyle, M.G. & Hardy, R.W.F. 1982. Biological nitrogen fixation. *In* Stevenson, F.J. (ed): *Nitrogen in Agricultural soils*, American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc Publisher Madison, Wisconsin USA. Agronomy, 22: 365-442.

Hensler, R.F., Olsen, R.J., Witzel, S.A., Altoe, O.J., Paulson, W.H. & Johannes, R.F. 1970. Effects of methods of manure handling on crop yields, nutrient recovery and runoff losses. *Trans. ASAE.*13: 726-731.

Hillel, D. (ed). 1982. *Introduction to Soil Physics*. Department of Soil Science, University of Massachusetts. Academic Press.

Ingesson, U. 1996. *Denitrifikationsprocessen -en litteratur studie*. Teknisk rapport nr 24 - Sveriges lantbruksuniversitet, Institutionen för markvetenskap, avd. för vattenvårdslära

ISCW, Institute Soil Climate Water, 2004.

Jansson, S.L. & Persson, J. 1982. Mineralisation and immobilization of soil nitrogen. *In* Stevenson, F.J. (ed): *Nitrogen in Agricultural soils*, American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc Publisher Madison, Wisconsin USA. Agronomy, 22: 229-252

Korentaljer, L. 1991. Agricultural use of sewage sludge - An overview. [Based on the paper by L. Korentaljer "A Review of the agricultural use of sewage sludge: benefits and potential hazards", Water SA 17:189-196 (1991)]. *In* Ekama, G.A., (ed): *Sewage Sludge Utilization and Disposal*, Water Institute of Southern Africa.

Legg, J.O. & Meisinger, J.J. 1982. Soil nitrogen budgets. *In* Stevenson, F.J. (ed): *Nitrogen in Agricultural soils*, American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc Publisher Madison, Wisconsin USA. Agronomy, 22: 503-566.

Leiros, M.C., Trasar-Cepeda, C., Seoane, S. & Gil-Sotres, F. 1999. Dependence of mineralisation of soil organic matter on temperature and moisture. *Soil Biology and Biochemistry* 31: 327-335.

Lötter, L.H. & Pitman, A.R. 1997. *Aspects of Sewage Sludge Handling and Disposal.* Report to the Water Research Commission by the Wastewater and Scientific Services Departments Johannesburg City Council. Report No 316/1/97

Novella, P.H. 1991. The disposal of wastewater sludge in sanitary landfills. *In* Ekama, G.A., (ed): *Sewage Sludge Utilization and Disposal*, Water Institute of Southern Africa.

Oberholster, G. 1983. South African practice in land disposal of sludge including legislation and health aspects – An overview. *Wat. Sci. Tech.* 15: 151-155.

Page, A.L. 1974. *Fate and Effects of Trace Elements in Sewage Sludge when Applied to Agricultural Lands - A Literature Review Study*. U.S. Environmental Protection Technology series. Report No. EPA-670/2-74-005. Department of Soil Science and Agricultural Engineering. University of California.

Page, A.L., Logan, T.J. & Ryan, J.A., 1987. *Land Application of Sludge-Food Implications* (ed). Lewis publisher, inc. United States of America.

Palmer, I.H. 1989. Long term options for sludge disposal in the Cape Town Metropolitan Area. *Paper presented at the 1st Biennial WISA* (Water Institute of South Africa) Conference. Cape Town, March 28, 1989. pp 16

Palmer, I.H. 1991. Using sludge on agricultural land, Palmer Development Group. *In* Ekama, G.A., (ed): *Sewage Sludge Utilization and Disposal*, Water Institute of Southern Africa.

Pratt, P.F. 1972. *Nitrate in the Unsaturated Zone under Agricultural Lands*. U.S. Environmental Protection Agency, Water Pollution Control Research Series, Project 16060 DOE 04/72. U.S. Govt. Printing Office, Washington, C.C.

Scanlon, R.B, Andraski, J.B & Bilskie, J. 2002. Miscellaneous methods for measuring matric or water potential. *In* Dane, H.J and Topp, C.G. (ed): *Methods of Soil Analysis*, Soil Science Society of America, Inc Publisher Madison, Wisconsin USA.
Agronomy 4(5): 662-665.

Schmidt, E.L. 1982. Nitrification in soil. *In* Stevenson, F.J. (ed): *Nitrogen in Agricultural Soils*, American Society of Agronomy, Inc., Crop Science Society of

America, Inc., Soil Science Society of America, Inc Publisher Madison, Wisconsin USA. Agronomy, 22: 253-288.

Smith, R. and Vasiloudis, H. 1989. *Inorganic Characterization of South African Municipal Sewage Sludges*. Water Research Commission Report No. 180/1/89. Pretoria. South Africa.179 pp.

Sparks, D.L. 1989. *Kinetics of Soil Chemical Processes.* College of Agricultural Science, Department of Plant Science, Delaware, pp 173-177.

Stevenson, F.J. 1982. Origin and distribution of nitrogen in soil. *In* Stevenson, F.J.(ed): *Nitrogen in Agricultural Soils*, American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc Publisher Madison, Wisconsin USA. Agronomy, 22: 1-39.

Tesfamariam, E.H. 2004. *Modelling the growth, development and soil water balance of Canola (Brassica napus L) (Hyolla 60)*, University of Pretoria, South Africa.

Van Niekerk, C.M. 2004. *Nitrogen Dynamics in Sewage Sludge and Commercial Fertilizer Enriched Soils*. Magister scienta, Department of Plant Production and Soil science, University of Pretoria.

Wennman, P. 2004. *Decomposition and Nitrogen Transformations in Digested Sewage Sludge Applied to Mine Tailings –Effects of Temperature, Soil Moisture, pH and Plants.* Licentiate thesis.

White, R.E. 1981. Pathways of phosphorus in soil. *In* T.W.G. Hucker., G.Catroux (ed): *Phosphorus in Sewage Sludge and Animal Waste Slurries*. Reidel, Dordrecht. Holland, pp. 21-46.

WHO, 1995. *Developing Human Health-Related Chemical Guidelines for Reclaimed Wastewater and Sewage Sludge Applications in Agriculture*. World Health Organisation, Geneva.

Williamson, J.C & Johnson, D.B. 1994. Laboratory assessment of nitrogen losses from restored topsoils at opencast mine sites. *Applied Soil Ecolog* 1: 53-63.

WRC. 1997. *Permissible Utilization and Disposal of Sewage Sludge. 1st Edition.* WATER RESEARCH COMMISSION. Department of Agriculture, Health, Water Affairs and Forestry, Water Institute of South Africa, Water Research Commission.. TT 85/97 Pretoria. TT 85/97 Pretoria.

Zucong, C., Laughlin, R.J. & Stevens, J. 2001. nitrous oxide and dinitrogen emissions from soil under different water regimes and straw amendment. *Chemosphere* 42:113-121.

Internet sources:

ACES (2005)
The Alabama Cooperative Extension System

http://www.aces.edu/department/crd/publications/ANR-607.html (2005-03-28)

Ag News (2005)
News and Public Affairs. Texas A & M.
University System Agricultural Program.
http://agnews.tamu.edu/drought/drghtpak98/drght19.html (2005-05-03)

Bengtsson (2004)
Microbial Mobilization and Immobilization of Soil Nitrogen.
Doctoral Dissertation. Lund.
http://www.lub.lu.se/cgi-bin/show_diss.pl/sci_684.html (2005-09-20)

Extension (2005)
Extension service, University of Minnesota
O'Leary, Rehm and Schmitt, 1994. Understanding Nitrogen in Soils.
http://www.extension.umn.edu/distribution/cropsystems/DC3770.html (2005-05-09)

Muextension (2005)
http://muextension.missouri.edu/explore/envqual/wq0254.htm (2005-05-04)

NASA (2005)
Goddar space flight centre
http://ltpwww.gsfc.nasa.gov/globe/NFTG/nitrocyc.htm (2005-05-04)

Naturvårdsverket (2002)
Rapport 5214
http://www.naturvardsverket.se/dokument/teknik/slam/pdf/5214.pdf (2004-12-12)

NMSU (2005)
New Mexico State University (2005)
http://weather.nmsu.edu/irrdoc/soilfc.html (2005-09-15)

Ohio State (2005)
The Ohio State University Extension Fact sheet
http://ohioline.osu.edu/aex-fact/0463.html (2005-08-23)

Rutgers (2005)
New Jersey Agricultural Experiment Station,
http://www.rce.rutgers.edu/pubs/pdfs/fs954.pdf (2005-01-04)

Soil Facts (2005)
Soil Science Society of North Carolina
http://www.soil.ncsu.edu/publications/Soilfacts/AG-439-03/ (2005-07-18)

Umass (2005)
University of Massachusetts Amherst
http://www.ecs.umass.edu/cee/reckhow/courses/seminar/Epsteintalk.html
(2005-03-28)

US EPA (2005a)
The United State Environmental Protection Agency
http://www.epa.gov/safewater/tcr/pdf/nitrification.pdf (2005-03-21)

U.S. EPA (2005b)
The United State Environmental Protection Agency
http://www.epa.gov/opptintr/pcb/effects.html#Other (2005-03-28)

APPENDIX 1: DETERMINATION OF AMMONIUM NITRATE.

Determination of the amount of ammonium nitrate added to 50 g of soil.

From the chemical properties we know that sludge contains 5.12% total N and the ammonium nitrate contains 35.15% inorganic N.

In Section 3.3.1 we calculated that the amount of sludge to be added to each 50 g of soil was 0.111 g. Then the equivalent amount of ammonium nitrate is:

Air-dried sludge ⇔ NH_4NO_3

$C_1 \quad * \quad M_1 \quad \Leftrightarrow C_2 \quad * \quad M_2$
$0.0512 * 0.111 \Leftrightarrow 0.3515 * X$
$0.0512 * 0.111 \Leftrightarrow 0.3515 * X$
$X \Leftrightarrow 0.0162$

The amount of ammonium nitrate is 0.0162 g per 50g of soil.

$M_{NO3NH4} = 80.21$ g / mol

0.111 g sludge / 50g soil ⇔ 0.0162 g NO_3NH_4 / 50g soil

To make the homogenization in the soil easier, ammonium nitrate was added as a liquid solution.
0.01617 / 80.21 = 0.20153 mmol * 2 = 0.4031 mmol

0.0162 g of ammonium nitrate was then equivalent to 0.2015 mmol, which is equivalent to 0.4031 mmol of N

So 0.4031 mmol of N was added to the samples.

APPENDIX 2*:* RECALCULATIONS OF N

The data obtained from the inorganic N results were in ml of HCl and to convert these to mg per kg soil we had to recalculate as:

$$C = \frac{(a - b) \cdot c \cdot d \cdot e}{m}$$

C = the concentration per kg soil (mg kg^{-1} soil)
a = volume of the sample (ml HCl)
b = volume of the blank (ml HCl)
c = concentration of the titration solution (Molar)
m = mass of soil (g)
d = molecular mass of N (g mol^{-1})
e = factor to get the answer in kg soil (1000)

$$17.86 = \frac{(4.47 - 1.28) \cdot 0.01 \cdot 14 \cdot 1000}{50}$$

So 4.47 ml of HCl gives the answer 17.86 mg kg^{-1} of inorganic N

APPENDIX 3: INORGANIC N VALUES

Inorganic N values for C, Sludge and AN in the soil (Blank sample 1.28 ml HCl).

Treatment	H_2O	Day	Value	Value	Value	Mean	Standard deviation
Appendix 3a.							
C	1	28	17.86	17.30	19.21	18.13	0.979
		21	17.42	8.74	13.66	13.27	4.353
		14	18.76	10.92	13.10	14.26	4.046
		7	17.47	12.77	9.18	13.14	4.157
		1	15.34	10.58	10.08	12.00	2.905
C	5	28	8.01	10.30	14.90	11.07	3.507
		21	7.06	18.76	10.98	12.26	5.957
		14	13.27	14.67	13.89	13.94	0.702
		7	10.75	15.62	5.99	10.79	4.816
		1	5.32	6.61	18.03	9.99	6.997
C	10	28	9.46	19.26	20.38	16.37	6.008
		21	10.47	15.62	8.57	11.55	3.650
		14	23.91	12.54	23.35	19.94	6.408
		7	9.91	18.82	11.87	13.53	4.679
		1	12.66	5.49	15.68	11.27	5.235
C	14	28	17.70	19.77	13.72	17.06	3.074
		21	14.45	9.74	11.48	11.89	2.379
		14	17.36	13.27	10.08	13.57	3.649
		7	24.25	11.87	16.02	17.38	6.300
		1	10.98	5.82	9.86	8.89	2.710
Appendix 3b.							
Sludge	1	28	45.47	49.00	36.74	43.74	6.314
		21	39.14	29.85	33.43	34.14	4.688
		14	35.34	27.05	30.41	30.93	4.169
		7	14.84	26.94	17.19	19.66	6.413
		1	12.21	15.34	19.15	15.57	3.477
Sludge	5	28	39.70	30.58	18.59	29.62	10.588
		21	24.02	24.92	14.34	21.09	5.869
		14	11.31	11.20	19.71	14.07	4.882
		7	11.31	11.93	12.49	11.91	0.588
		1	12,38	14,39	11,98	12,92	1,292
Sludge	10	28	24.75	44.18	14.50	27.81	15.075
		21	15.29	13.55	17.64	15.49	2.052
		14	14.90	18.26	13.38	15.51	2.494
		7	13.66	24.42	12.38	16.82	6.611

		1	11.37	23.69	17.08	17.38	6.165
Sludge	14	28	22.46	27.05	20.66	23.39	3.293
		21	22.62	18.20	24.42	21.75	3.200
		14	44.86	12.26	25.31	27.48	16.404
		7	19.54	17.58	18.31	18.48	0.991
		1	15.01	14.00	50.18	26.39	20.601
Appendix 3c.							
AN	1	28	165.54	126.11	152.32	147.99	20.066
		21	182.06	175.50	211.46	189.67	19.148
		14	195.27	50.12	123.31	122.90	72.577
		7	188.50	181.05	198.63	189.39	8.826
		1	174.38	207.87	200.87	194.38	17.664
AN	5	28	134.68	137.76	147.34	139.93	6.600
		21	179.82	187.77	199.92	189.17	10.125
		14	166.60	232.18	145.77	181.51	45.093
		7	174.38	196.34	196.45	189.06	12.706
		1	204.62	210.50	208.66	207.93	3.007
AN	10	28	160.61	121.52	92.74	124.95	34.066
		21	162.40	111.22	168.11	147.24	31.330
		14	171.42	150.02	128.46	149.97	21.476
		7	164.53	216.44	213.98	198.31	29.286
		1	182.67	220.14	201.88	201.56	18.734
AN	14	28	107.63	150.75	154.17	137.52	25.938
		21	162.12	217.84	140.78	173.58	39.786
		14	166.82	171.19	177.58	171.86	5.407
		7	210.78	174.10	205.02	196.63	19.724
		1	142.69	204.18	208.77	185.21	36.897

APPENDIX 4: pH DATA

Measured pH values for C, Sludge and AN treatments in the soil.

Treatment	H_2O	Day	Value	Value	Mean value	Standard deviation
Appendix 4a.						
C	1	28	5.67	5.71	5.69	0.028
		21	5.85	5.60	5.73	0.177
		14	5.61	5.66	5.64	0.035
		7	5.65	5.52	5.59	0.092
		1	5.88	5.62	5.75	0.184
C	5	28	5.80	5.66	5.73	0.099
		21	5.74	5.70	5.72	0.028
		14	5.57	5.68	5.63	0.078
		7	5.50	5.55	5.53	0.035
		1	5.67	5.52	5.60	0.106
C	10	28	5.72	5.70	5.71	0.014
		21	5.70	5.61	5.66	0.064
		14	5.62	5.72	5.67	0.071
		7	5.79	5.69	5.74	0.071
		1	5.36	5.81	5.59	0.318
C	14	28	5.69	5.65	5.67	0.028
		21	5.69	5.55	5.62	0.099
		14	5.74	5.55	5.65	0.134
		7	5.78	5.55	5.67	0.163
		1	5.34	5.58	5.46	0.170
Appendix 4b**.**						
Sludge	1	28	5.95	5.82	5.89	0.092
		21	5.89	5.76	5.83	0.092
		14	5.72	5.73	5.73	0.007
		7	5.90	5.73	5.82	0.120
		1	5.72	5.22	5.47	0.354
Sludge	5	28	5,82	5,70	5,76	0,085
		21	5.74	5.65	5.70	0.064
		14	5.70	5.55	5.63	0.106
		7	5.71	5.70	5.71	0.007
		1	5.71	5.68	5.70	0.021
Sludge	10	28	5.89	5.73	5.81	0.113
		21	5.85	5.51	5.68	0.240
		14	5.70	5.70	5.70	0.000
		7	5.36	5.75	5.56	0.276

		1	5.74	5.47	5.61	0.191
Sludge	14	28	5.86	5.76	5.81	0.071
		21	5.70	5.58	5.64	0.085
		14	5.76	5.74	5.75	0.014
		7	5.66	5.68	5.67	0.014
		1	5.74	5.70	5.72	0.028

Appendix 4c.

AN	1	28	4.94	4.83	4.89	0.078
		21	5.04	5.12	5.08	0.057
		14	4.87	4.87	4.87	0.000
		7	5.39	5.00	5.20	0.276
		1	5.35	5.23	5.29	0.085
AN	5	28	4.96	4.89	4.93	0.049
		21	4.90	4.96	4.93	0.042
		14	4.90	5.12	5.01	0.156
		7	5.26	5.30	5.28	0.028
		1	5.31	5.21	5.26	0.071
AN	10	28	4.79	5.04	4.92	0.177
		21	5.02	5.00	5.01	0.014
		14	5.04	4.93	4.99	0.078
		7	5.28	5.26	5.27	0.014
		1	4.91	5.36	5.14	0.318
AN	14	28	4.93	5.02	4.98	0.064
		21	5.02	5.09	5.06	0.049
		14	5.00	4.99	5.00	0.007
		7	5.31	5.27	5.29	0.028
		1	5.28	5.26	5.27	0.014

APPENDIX 5*:* WATER POTENTIAL DATA

Water potential data in MPa for C, Sludge and AN treatments in the soil.

Treatment	H_2O	Day	Value	Value
Appendix 5a.				
C	1	28	-16.99	
		21	-16.65	
		14	-12.38	
		7	-12.98	
		1	-3.46	
C	5	28	-29.48	
		21	-21.26	
		14	-15.18	
		7	-15.85	
		1	-8.01	
C	10	28	-24.65	
		21	-19.00	
		14	-16.86	
		7	-17.77	
		1	-4.56	
C	14	28	-22.82	
		21	-31.43	
		14	-15.63	
		7	-20.74	
		1	-15.06	
Appendix 5b.				
Sludge	1	28	-18.94	
		21	-16.69	
		14	-12.48	
		7	-6.84	
		1	-2.75	
Sludge	5	28	-30.88	
		21	-27.02	
		14	-19.38	
		7	-23.54	
		1		
Sludge	10	28	-21.83	
		21	-25.45	
		14	-20.27	
		7	-16.35	
		1	-11.08	
Sludge	14	28	-22.67	
		21	-47.57	
		14	-15.89	-16.09

		7	-19.02	
		1	-13.88	

Appendix 5c.

AN	1	28	-19.94	-19.44
		21	-30.05	-8.2
		14	-3.22	-5.34
		7	-2.96	
		1	-2.05	
AN	5	28	-19.60	-28.92
		21	-27.24	-22.56
		14	-24.50	-20.35
		7	-10.61	
		1	-2.91	
AN	10	28	-42.44	-28.83
		21	-20.73	-16.9
		14	-13.76	-4.54
		7	-8.52	
		1	-4.48	
AN	14	28	-38.24	-31.54
		21	-31.46	-24.31
		14	-14.19	-16.09
		7	-6.10	
		1	-4.77	

APPENDIX 6: STATISTICS, INORGANIC N

6a) Statistics from Inorganic N measurements, C

```
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  40  "General Analysis of Variance."
  41  BLOCK "No Blocking"
  42  TREATMENTS Days,H2O,Rep,Days*H2O
  43  COVARIATE "No Covariate"
  44        ANOVA    [PRINT=aovtable,means;    FACT=32;    FPROB=yes;
PSE=diff,lsd,means; LSDLEVEL=5] CtlTotN


***** Analysis of variance *****

Source of variation        d.f.   s.s.       m.s.     v.r.   F pr.
Days                        4     225.02      56.26    3.19   0.026
H2O                         3      77.05      25.68    1.46   0.245
Rep                         2      14.33       7.17    0.41   0.670
Days.H2O                   12     182.97      15.25    0.86   0.589
H2O.Rep                     6     216.41      36.07    2.04   0.088
Residual                   32     564.65      17.65
Total                      59    1280.42
```

6b) Statistics from Inorganic N measurements, Sludge

```
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***** Analysis of variance *****

Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Days                        4    1535.78     383.94    5.97  0.001
H2O                         3    1148.35     382.78    5.95  0.002
Rep                         2      20.28      10.14    0.16  0.855
Days.H2O                   12    1161.42      96.78    1.50  0.174
H2O.Rep                     6     600.17     100.03    1.55  0.193
Residual                   32    2059.53      64.36
Total                      59    6525.53
```

6c) Statistics from Inorganic N measurements, AN

```
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***** Analysis of variance *****

Source of variation     d.f.       s.s.       m.s.    v.r.  F pr.
Days                       4    30296.6     7574.1    8.99  <.001
H2O                        3     2384.5      794.8    0.94  0.431
Rep                        2      155.9       78.0    0.09  0.912
Days.H2O                  12     9167.1      763.9    0.91  0.551
H2O.Rep                    6     6136.3     1022.7    1.21  0.325
Residual                  32    26956.9      842.4
Total                     59    75097.4
```

APPENDIX 7: STATISTICS, pH

7a) Statistics from pH measurements, C

```
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  52  "General Analysis of Variance."
  53  BLOCK "No Blocking"
  54  TREATMENTS Days,Water,Rep,Days*Water
  55  COVARIATE "No Covariate"
  56   ANOVA  [PRINT=aovtable,information,means;  FACT=32;  FPROB=yes;
PSE=diff,lsd,means; LSDLEVEL=5]\
  57   CTLpH

***** Analysis of variance *****

Source of variation       d.f.    s.s.       m.s.     v.r.  F pr.
Days                        4    0.05317    0.01329    0.86  0.507
Water                       3    0.02778    0.00926    0.60  0.624
Rep                         1    0.01369    0.01369    0.89  0.360
Days.Water                 12    0.13154    0.01096    0.71  0.721
Water.Rep                   3    0.04397    0.01466    0.95  0.439
Residual                   16    0.24644    0.01540
Total                      39    0.51660


* MESSAGE: the following units have large residuals.

*units* 37         -0.191   s.e. 0.078
*units* 38          0.191   s.e. 0.078


***** Tables of means *****

Grand mean  5.650
```

7b) Statistics from pH measurements, Sludge

```
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                  GenStat Seventh Edition (SP1)
                  GenStat Procedure Library Release PL15
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```

```
Sludge pH

***** Analysis of variance *****

Variate: pH Sludge

Source of variation  d.f.(m.v.) s.s.     m.s.    v.r.   F pr.
Days                  4         0.17014  0.04254  2.36  0.104
Water                 3         0.03110  0.01037  0.58  0.641
Rep                   1         0.10375  0.10375  5.75  0.031
Days.Water           12         0.17570  0.01464  0.81  0.637
Water.Rep             3         0.02425  0.00808  0.45  0.722
Residual             14(2)      0.25241  0.01803
Total                37(2)      0.74903


* MESSAGE: the following units have large residuals.

*units* 29         -0.233   s.e. 0.079
*units* 30          0.233   s.e. 0.079


***** Tables of means *****

Variate: pH Sludge

Grand mean  5.705
```

7c) Statistics from pH measurements, AN

```
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 111  "General Analysis of Variance."
 112  BLOCK "No Blocking"
 113  TREATMENTS Days,Water,Rep,Days*Water
 114  COVARIATE "No Covariate"
 115   ANOVA [PRINT=aovtable,information,means; FACT=32; FPROB=yes;
PSE=diff,lsd,means; LSDLEVEL=5]\


***** Analysis of variance *****

Source of variation     d.f.   s.s.     m.s.       v.r.   F pr.
Days                     4    0.78517   0.19629   14.90   <.001
Water                    3    0.01909   0.00636    0.48   0.699
Rep                      1    0.00156   0.00156    0.12   0.735
Days.Water              12    0.08092   0.00674    0.51   0.878
Water.Rep                3    0.06091   0.02030    1.54   0.242
```

```
Residual                  16    0.21078    0.01317
Total                     39    1.15844


* MESSAGE: the following units have large residuals.

*units* 37         -0.170   s.e. 0.073
*units* 38          0.170   s.e. 0.073


***** Tables of means *****

Variate: pH Ammonium nitrate

Grand mean 5.081
```

7d) Statistics from pH measurements, all three treatments together

```
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***** Analysis of variance *****

Variate: pH

Source of variation  d.f.(m.v.)   s.s.     m.s.       v.r.     F pr.
Days                   4         0.09628    0.02407     1.56     0.201
H2O                    3         0.01381    0.00460     0.30     0.826
Trt                    2         9.54148    4.77074   309.25     <.001
Reps                   1         0.05328    0.05328     3.45     0.070
Days.H2O              12         0.06329    0.00527     0.34     0.976
Days.Trt               8         0.91250    0.11406     7.39     <.001
H2O.Trt                6         0.06417    0.01069     0.69     0.656
H2O.Reps               3         0.10776    0.03592     2.33     0.087
Trt.Reps               2         0.06587    0.03294     2.13     0.130
Days.H2O.Trt          24         0.32464    0.01353     0.88     0.628
H2O.Trt.Reps           6         0.02133    0.00356     0.23     0.965
Residual              46(2)      0.70963    0.01543
Total                117(2)     11.91167


***** Tables of means *****

Variate: pH


      Trt        1        2        3
             5.650    5.705    5.081
```