

**A GEOCHEMICAL INVESTIGATION OF THE AQUATIC  
SEDIMENTS, GROUNDWATER AND SURFACE WATER  
OF THE VERLORENVLEI COASTAL LAKE, WITH  
SPECIAL REFERENCE TO NITRATE  
TRANSFORMATIONS.**

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**I declare that this research is my own work and that it was conducted under the supervision of Assoc. Prof. J.P. Willis and Dr. M.V. Fey. No part of this research has been submitted in the past, or is being submitted, for a degree at another university.**

**T.R. Harck**

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## ABSTRACT

The incorporation of nitrogen in living cells gives rise to cycling between atmospheric, inorganic and organic forms of nitrogen. Nitrogen cycling is largely controlled by microbial respiration and metabolism. In aquatic systems, N-cycling occurs dominantly in sediments.

Removal of nitrogen from aquatic sediments occurs through the successive N-transformation processes of mineralisation (organic N  $\rightarrow$   $\text{NH}_4^+$ ), nitrification ( $\text{NH}_4^+ \rightarrow \text{NO}_3^-$ ) and denitrification ( $\text{NO}_3^- \rightarrow \text{N}_2$ ). Denitrification, mineralisation and also immobilisation of inorganic N ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ) to organic N occur under reducing conditions.

Build-up of the nitrate ( $\text{NO}_3^-$ ) concentration in groundwater is a widely-recognised phenomenon. Groundwater nitrate may contribute significantly to the N input to aquatic bodies that receive groundwater flow. The Verlorenvlei coastal lake on the arid south-west coast of South Africa is an important ecological habitat and also a valuable agricultural water resource.

Analyses conducted in this study indicate that the groundwater, which flows towards the lake, has a significantly higher  $\text{NO}_3^-$  concentration than the lake water. The difference in  $\text{NO}_3^-$  concentration is due to:

1. Dilution of groundwater by a larger quantity of low-nitrate water in the lake, and/or,
2. Removal of nitrate from groundwater through microbial processes in the lake sediments.

Insufficient information is available concerning the magnitude of groundwater flow into the lake to investigate the importance of option 1. Experiments were conducted on two lake sediments (high organic content and low organic content) to determine the relevance of option 2.

Sediment subsamples were incubated under reducing conditions and amended with 25mg/l and 100mg/l  $\text{NaNO}_3$ -N solutions with and without the addition of 40mg/l glucose and with and without irradiation. After 10 days incubation, a period chosen to simulate the residence time of groundwater in the sediments, the added  $\text{NO}_3^-$  had all been removed through denitrification or immobilised as organic N. The exact removal pathway could not be determined. The high organic content sediment was found to remove nitrate more efficiently.

It was concluded that the decrease in nitrate concentration as high-nitrate groundwater flows into the Verlorenvlei lake can probably be ascribed to denitrification or immobilisation processes under reducing conditions in the sediment lining of the lake.

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## INTRODUCTION

Interest in the Verlorenvlei coastal lake is largely limited to three groups of people: those who profess a desire to shield this unique ecosystem from anthropogenic impacts; those who rely, to a greater or lesser degree, on the ecosystem for their livelihood; and the authorities responsible for its management. Numerous authorities are (theoretically) responsible for the Verlorenvlei lake but, whatever management strategy there is, has been poorly implemented. Eventually, a more responsible attitude will need to be adopted by all three groups in order to maintain the status of the lake as an ecological wonder and as a useful resource. In the meantime, the lake can be comprehensively studied in order to catalogue its vulnerabilities and assemble a database of relevant historical information and research data that can be used in the effective management of the lake system. The present study is intended to contribute data concerning the nitrogen geochemistry of the Verlorenvlei lake to the database of relevant information.

The Verlorenvlei lake lies in an area of intensive agricultural activity and is, to a large degree, the reason for that activity since it is an important source of water in an otherwise arid area. Geochemically, however, lakes are reservoirs that are the semi-permanent resting places for the components of runoff and water flow from their catchments.

Nitrogen is an important nutrient element that strongly affects not only the growth of crops, but also the productivity of aquatic ecosystems. Hence, the very real possibility exists that the application of fertiliser nitrogen to attain the crop yields desired by farmers in the Verlorenvlei lake catchment may increase the input of nitrogen to the lake system. Increasing the supply of this important nutrient may result in eutrophication and its degrading effects on water quality and the non-plankton aquatic life of the lake. Nitrate is one of the most mobile forms of nitrogen. Groundwater flow is a potentially major source of nitrate input to aquatic bodies and merits serious consideration in the study of nitrogen enrichment of the Verlorenvlei, particularly since surface runoff and water flow is limited in the Verlorenvlei catchment.

In this dissertation, Chapter 1 presents a short review of pertinent literature regarding the geochemistry of nitrogen and its cycling in the aquatic environment, especially in aquatic sediments. It is apparent from the literature that nitrogen cycling is mediated to a very large extent by the activities of microbes in sediments and water. Chapter 2 comprises a brief description of the Verlorenvlei lake and the questions which were formulated to maintain the focus of this project.

A programme of groundwater and surface water collection was undertaken at the Verlorenvlei and this is described in Chapter 3. The analysis of these samples with respect to major ions, including nitrate, is also described, and the results are presented in a form that illustrates the conclusions drawn regarding the geochemistry of these waters.

In Chapter 4, the sampling of two sediments from the Verlorenvlei lake is summarised and the findings of a series of experiments to investigate the nitrate removal capacity of these sediments is outlined. The implications of the experimental results for the Verlorenvlei lake are also discussed.

In Chapter 5, a short summary of the salient deductions arising from this research is presented with recommendations for future research.

# Chapter 1 - LITERATURE REVIEW: NITRATE REDUCTION IN AQUATIC SEDIMENTS

## 1.1. Introduction

Nitrogen is a nutrient element in terrestrial biology. Excess input of nitrogen (with or without phosphorus) to aquatic systems has been linked to changes in aquatic ecology described as "eutrophication". Although the eutrophic (Greek: well-nourished) state is a natural condition attained by many lakes, man's use of the water from such systems is constrained by its low dissolved oxygen content and the abundance of suspended micro-organisms.

However, anthropogenically-elevated nitrogen inputs to aquatic systems are increasing as the size of the human population grows. It has become necessary to investigate the fate of nitrogen in aquatic systems to understand how these systems will react to increased nutrient loading.

The following literature review describes nitrogen geochemistry and sources of nitrogen in aquatic systems; and the significance of sediments as the primary host of nitrogen-cycling processes is explored. The processes of nitrogen-cycling are reviewed with an emphasis on denitrification, one of the mechanisms by which excess nitrogen is removed from aquatic systems. Pertinent literature regarding the increase in nitrogen-pollution of groundwater and its implications to nitrogen cycling in aquatic systems is discussed. The findings of the review are summarised in the conclusion.

## 1.2. Nitrogen geochemistry

Three isotopic forms of nitrogen are known:  $^{13}\text{N}$ ,  $^{14}\text{N}$ , and  $^{15}\text{N}$ . Nitrogen-13 is radioactive with a half-life of ~10 minutes and  $^{14}\text{N}$  makes up 99.63% of total nitrogen (Stevenson, 1972).

Table 1.1. Common N-species and their oxidation states.

Oxidation state	-3	0	+1	+3	+5
Nitrogen species	$\text{NH}_3$ $\text{NH}_4^+$	$\text{N}_2$	$\text{N}_2\text{O}$	$\text{NO}_2^-$	$\text{NO}_3^-$

Nitrogen is an important element in reactions in living organisms and, after C, H and O is a major component of living cells. The incorporation of N in organic matter gives rise to a complex cycling of N between different forms. The element changes its oxidation state during the cycling process (Table 1.1.).

The molecular species  $N_2$  is the most common gas in the atmosphere and is extremely stable and unreactive. In water, the dominant N-species are  $NO_3^-$ ,  $NH_4^+$ ,  $NO_2^-$  and N-containing organic matter. Nitrite ( $NO_2^-$ ) is generally found at low concentrations and is often not detected in water. Ammonium ( $NH_4^+$ ) is the dominant N-species in aquatic sediments.

### 1.2.1. Sources of nitrogen in aquatic systems

Nitrogen may enter an aquatic system in at least four ways:

- 1) Mineralisation of organically bound nitrogen through decomposition of organic matter;
- 2) Riverine input to the system;
- 3) Input from polluted groundwater in hydrogeological continuity with the system;
- 4) Direct N input to the water body by, for example, sewage or precipitation of N-species in rain.

### 1.3. The nitrogen cycle in water and sediment

Since water covers approximately 75% of the planet, nutrient cycling between sediments and the overlying water column is of prime importance in the generation and sustenance of life. The concentrations of nitrogen species is dependant on the rates of various nitrogen-cycling processes. These processes are illustrated in Figure 1.1 and defined below.

- 1) **Fixation** - Reduction of  $N_2$  to organic N.
- 2) **Assimilation/Assimilatory Reduction/Immobilisation** - Absorption of inorganic N ( $NO_3^-$ ,  $NO_2^-$ ,  $NH_4^+$ ) by living organisms and incorporation into cell biomass.
- 3) **Mineralisation/Ammonification** - Decomposition of organically-bound N to  $NH_4^+$ .
- 4) **Nitrification** - Oxidation of  $NH_4^+$  to  $NO_3^-$ .
- 5) **Denitrification** - Reduction of  $NO_3^-$  to  $N_2O$  and  $N_2$ .
- 6) **Dissimilatory reduction** - Reduction of inorganic N species, especially  $NO_3^-$ , to inorganic N, especially  $NH_4^+$ .

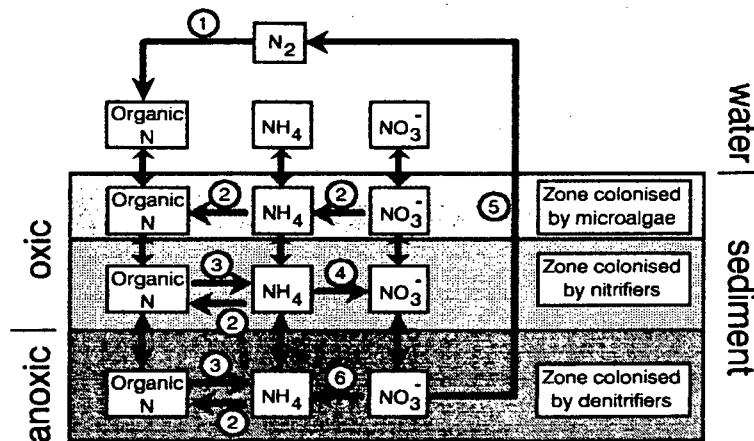


Figure 1.1 Schematic cross-section through sediment-water interface illustrating the various N-cycling processes and their theoretical locations in sediment layers (after Rysgaard *et al.*, 1993).

All of these processes are catalysed and mediated by microbes. Microbial activities are mainly responsible for N turnover in lake sediments (Forsberg, 1989). Since the number of bacteria in sediments is orders of magnitude greater than in the water column, N cycling is most significant in sediments. A number of chemical, mechanical and physical factors affect the net uptake or release of nitrogen at the sediment-water interface, mainly by influencing the metabolism of microbial populations.

## 1.4. Processes in the nitrogen cycle

### 1.4.1. Nitrogen fixation

Most nitrogen on earth is present as biologically unavailable  $N_2$ . Prokaryotic organisms can fix nitrogen from the atmosphere utilising an enzyme called nitrogenase. Photoautotrophic cyanobacteria (blue-green algae), photoautotrophic anaerobic bacteria, aerobic and anaerobic heterotrophic bacteria and chemoautotrophic bacteria are among the organisms in which fixation occurs (Howarth *et al.*, 1988b). In a review of experimentally-determined fixation rates Howarth *et al.* (1988b) found that planktonic nitrogen fixation contributed 0 to 82% of the total N input to various aquatic systems.

Nitrogen fixation is inhibited by high concentrations of  $NO_3^-$  and  $NH_4^+$  which suppress the synthesis of nitrogenase. Since  $NH_4^+$  is usually found in greater concentrations in sediments than in the water column, N-fixation in aquatic sediments is generally low (Howarth *et al.*, 1988a) (Table 1.2).

Low N-fixation rates in estuarine and marine environments are ascribed to the lower availability of trace elements in marine waters (Table 1.2). Iron and molybdenum are necessary elements in the synthesis of nitrogenase. Sulphate inhibits molybdate assimilation by planktonic algae and bacteria due to its steric similarity with molybdate (Howarth *et al.*, 1988a).

Physical factors can also affect N-fixation. The penetration and quality of light affect the N-fixing capability of cyanobacteria; stratification and mixing in lake waters can change the  $\text{NH}_4^+$  concentration; calm conditions favour the development of algal blooms; and the concentration of dissolved  $\text{O}_2$  affects nitrogenase activity and the speciation of Mo. Macrophyte beds may provide a substrate for bacteria and algae and thus enhance nitrogen fixation.

Table 1.2. Rates of N-fixation in various aquatic environments (after Howarth *et al.*, 1988b).

System	Range of N-fixation rates ( $\text{g N m}^{-2} \text{ yr}^{-1}$ )
Oceans	0.002 - 0.09
Estuaries	0.013 - 1.2
Oligotrophic Lakes	0.0003 - 2.0
Mesotrophic Lakes	0.013 - 0.094
Eutrophic Lakes	0.20 - 9.2

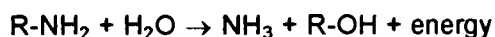
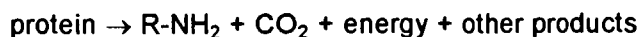
#### 1.4.2. Assimilation

Bacteria preferentially utilise  $\text{NH}_4^+$  as a source of nitrogen for the synthesis of biomass. If  $\text{NH}_4^+$  is not present in sufficient quantities, other N-species must be utilised. In pure cultures of micro-organisms, this requires the reduction of species such as  $\text{NO}_3^-$  and  $\text{NO}_2^-$  (Payne, 1973). Reduction is achieved through reductase enzymes. Nitrate reductase can be synthesised under aerobic or anaerobic conditions, although the form of the reductase varies depending on the conditions (Payne, 1973).

The assimilation of  $\text{NH}_4^+$ , or other inorganic species that have been reduced to ammonium, is related to the nitrogen:carbon ratio of the substrate and the nitrogen:carbon ratio in the bacterial cells. Observations in marine sediments indicate that, if carbon is in excess in the substrate, then assimilation of  $\text{NH}_4^+$  is rapid and all available  $\text{NH}_4^+$  is used up (Blackburn and Henriksen, 1983). Similar observations have been made in soils amended with glucose to increase the carbon content of the substrate (Stanford *et al.*, 1975).

### 1.4.3. Ammonification

Decomposition of organic matter occurs through the activities of heterotrophic microbes which utilise organic carbon compounds as an energy source. This process occurs under oxic and anoxic conditions and results in the release of nitrogen compounds from organic species, e.g. protein. This is schematically shown by the reactions:



The ammonia molecule subsequently gains a proton to form ammonium ( $\text{NH}_4^+$ ) in the aquatic environment.

### 1.4.4. Nitrification

The nitrification of ammonium to nitrite and nitrate in marine and estuarine sediments is illustrated in Figure 1.2. Radioactively labelled  $^{15}\text{NH}_4^+$  was added to laboratory samples and the increasing quantity of  $^{15}\text{N}$  in  $\text{NO}_2^- + \text{NO}_3^-$  with time is recorded in the experimental results of Nishio *et al.* (1983).

The experimental results of Rysgaard *et al.* (1993) indicate that nitrification rates are higher in estuaries than in lakes. Nitrification is restricted to the upper sediments in estuaries, more specifically, to the oxic layer which only reaches to a depth of 1-3mm below the sediment water interface, and in the immediate vicinity of burrows (Blackburn and Henriksen, 1983; Rysgaard *et al.*, 1993).

Nitrification requires high redox potentials and high dissolved  $\text{O}_2$  concentrations. In marine sediments, the injection of  $\text{O}_2$  rich water by burrowing benthic fauna has been cited as a mechanism for nitrification at depths of 20-30cm below the sediment-water interface (Grundmanis and Murray, 1977). The autotrophic microbes responsible for nitrification react quickly to changes in oxygen availability and can survive long periods of anaerobic conditions (Jensen *et al.*, 1993).

Nitrification rates are controlled by the rate of  $\text{NH}_4^+$  diffusion into the sediment. Enrichment of  $\text{NH}_4^+$  in estuarine sediments stimulates nitrification (Nishio *et al.*, 1983). Higher temperatures have been found to have the same effect (Klapwijk and Snodgrass, 1982).

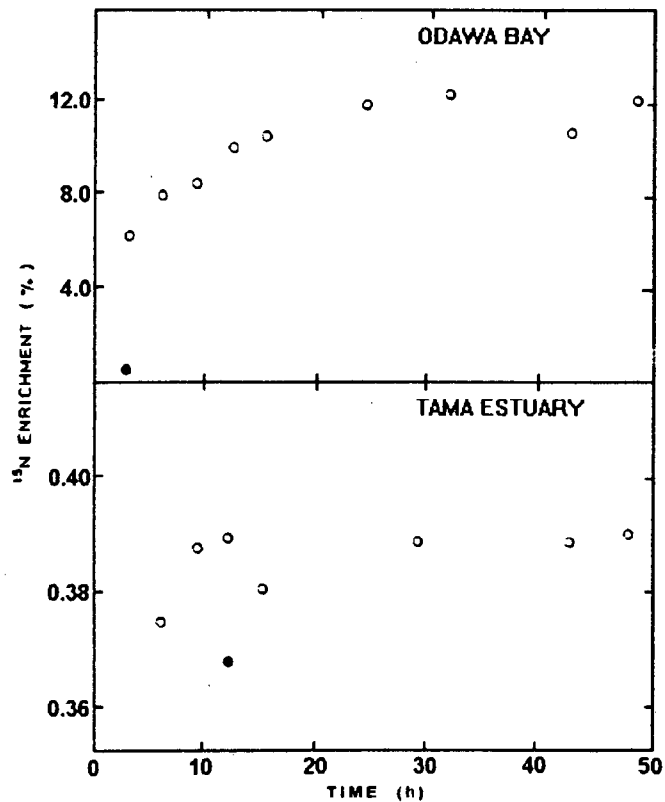


Figure 1.2. Enrichment with time of  $^{15}\text{N}$  in nitrate and nitrite produced from  $^{15}\text{N}$  amended ammonium added to sediments. Filled circle indicates enrichment % in ammonium. Open circles represent enrichment in nitrate + nitrite (from Nishio *et al.*, 1983).

Nitrification is coupled to denitrification, especially when nitrification occurs in close proximity to oxic-anoxic boundaries (Jensen *et al.*, 1993). The relative rates of these reactions will control the nitrification rate. However, Rysgaard *et al.* (1993) stress that results obtained under laboratory conditions may be in error since changes in pH,  $\text{NH}_4^+$  concentration,  $\text{O}_2$ , and  $\text{CO}_2$  will probably result in differing nitrification rates in *in situ* sediments.

#### 1.4.5. Denitrification

Denitrification is the only biochemical process which reduces  $\text{NO}_3^-$  and  $\text{NO}_2^-$  to gaseous  $\text{N}_2$  and  $\text{N}_2\text{O}$  (Nishio *et al.*, 1983) (Figure 1.3). The sequence  $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$  operates in biochemical denitrification. The process is a result of microbial utilisation of nitrate in respiration under anaerobic conditions. Various genera of denitrifying bacteria have been identified (Table 1.3.).

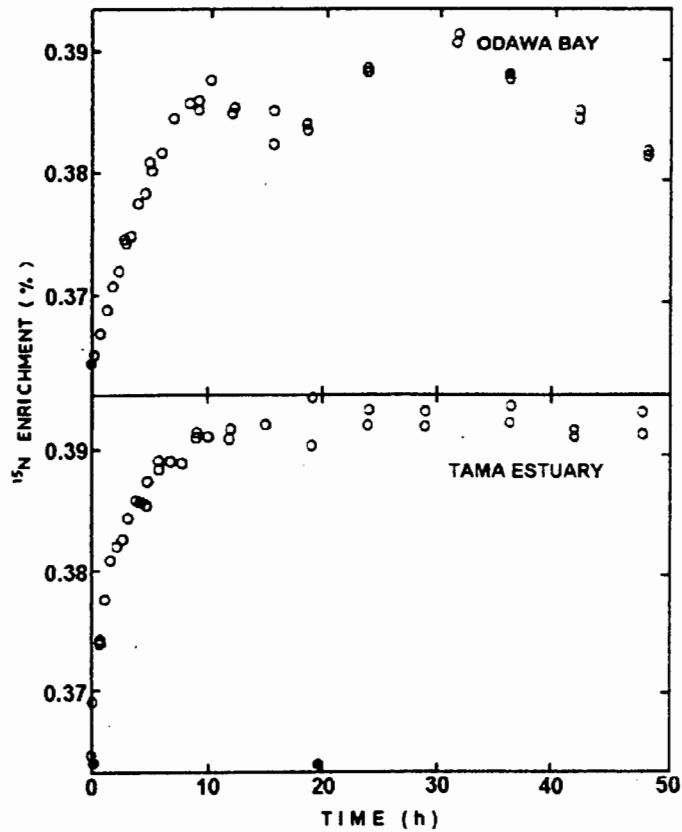


Figure 1.3. Enrichment of  $^{15}\text{N}$  in nitrogen gas evolved from a sediment water system to which  $^{15}\text{N}$  amended nitrate has been added. Open circles represent enrichment % in nitrogen gas. Filled circles represent  $^{15}\text{N}$  enrichment in nitrate (from Nishio *et al.*, 1983).

Table 1.3. Genera of denitrifying bacteria (from Payne, 1973).

1) <i>Achromobacter</i>	9) <i>Moraxella</i>
2) <i>Alcaligenes</i>	10) <i>Nitrosomonas</i>
3) <i>Bacillus</i>	11) <i>Propionibacterium</i>
4) <i>Chromobacterium</i>	12) <i>Pseudomonas</i>
5) <i>Corynebacterium</i>	13) <i>Spirillum</i>
6) <i>Halobacterium</i>	14) <i>Thiobacillus</i>
7) <i>Hyphomicrobium</i>	15) <i>Xanthomonas</i>
8) <i>Micrococcus</i>	

The development of nitrate reductase, the enzyme utilised by bacteria to achieve nitrate reduction, is affected by several factors. In pure cultures, the quantity of reductase produced is related to the concentration of nitrate and is inversely related to the oxygen concentration (Payne, 1973). No reductase is formed unless nitrate is present. The presence of azide, a bacteriostat, can actually induce nitrate reductase production in some cultures (Payne, 1973). Incubation temperatures around 30°C and a neutral or slightly alkaline pH generally favour the growth of denitrifying bacteria (Payne, 1973).

Denitrification is the major mechanism of N removal from shallow, turbid estuaries with riverine nitrate inputs (Smith *et al.*, 1985). The relative magnitude of denitrification in different aquatic systems is uncertain due to the limited numbers of studies which have quantified denitrification rates, but it is apparent that denitrification removes a larger percentage of N in freshwater systems than in marine systems and denitrification is greatest in sediments (Barnes *et al.*, 1975; Seitzinger, 1988) (Table 1.4).

Table 1.4. Ranges of denitrification rates for various aquatic systems and proportion of sediment-water N flux due to denitrification (after Seitzinger, 1988)

	denitrification rate ( $\mu\text{mol N m}^{-2} \text{h}^{-1}$ )	sediment-water $\text{N}_2$ flux as percentage of total N flux
River/streams	0 - 345	78 -100
Lakes		
oligotrophic	5 - 56	
moderately eutrophic	2 - 25	(all lakes) 77 -100
eutrophic	42 - 171	
Coastal marine	0 -1067	15 -75

Denitrification occurs in the presence of organic matter (Mackin and Swider, 1989). During decomposition, facultative anaerobic bacteria utilise nitrite or nitrate as the terminal electron acceptor during oxidation of organic carbon (Keeney, 1973; Grundmanis and Murray, 1977; Seitzinger, 1988). The availability of easily oxidised organic matter is cited as one of the most important controlling factors in denitrification and results in a high degree of correlation between water-soluble carbon contents of sediments and denitrification rates (Hill and Sanmugadas, 1985).

Denitrification occurs under conditions of low ( $\leq 0.2 \text{mg l}^{-1}$ ) dissolved  $\text{O}_2$  concentration. The burrowing and ventilating activities of benthic fauna maintain an anoxic but relatively high redox potential zone around their burrows which has been associated with increased rates of denitrification (Binnerup *et al.*, 1992). Increase of  $\text{O}_2$  concentration due to benthic photosynthesis during the day has been found to inhibit denitrification, which leads to a diurnal variation in the denitrification capacity of some sediments in clear and/or shallow waters (Andersen *et al.*, 1984). Seitzinger (1988) speculated that the greater efficiency of removal of mineralized N via denitrification in freshwater sediments compared to marine sediments is due to differences in the  $\text{O}_2$  solubility in fresh water and salt water. Turbulence is important for the release of  $\text{N}_2$  produced by denitrification from sediments (Jensen *et al.*, 1984).

High redox potentials favour denitrification (Kaspar, 1983). Nitrate is an electron acceptor so, as  $\text{NO}_3^-$  increases, the redox potential and the denitrification rate increase also (Jorgensen, 1989).

Nishio *et al.* (1983) noted that a high concentration of  $\text{NO}_3^-$  in the overlying water stimulated denitrification rates in sediments. Oremland *et al.* (1984) confirmed this and noted that denitrification was limited to the upper few centimetres of sediment. Denitrification potential exists below this level but if  $\text{NO}_3^-$  comes from the water column then it never penetrates below a certain depth without being denitrified. Consequently, denitrification is found to decrease with depth. Andersen *et al.* (1984) noted the highest denitrification rates in the upper 1cm of sediments and a decline with decreasing  $\text{NO}_3^-$  concentration. Macrophytic vegetation affects the distribution of  $\text{NO}_3^-$  in sediments through assimilation (Seitzinger, 1988). High denitrification rates in sandy sediments have been attributed to the higher porosity of sands facilitating migration of nitrate to denitrifying zones (Kaspar, 1983; Yoon and Benner, 1992).

Denitrification is inhibited by free sulphide (Jorgensen, 1989). This is at least partly due to the inhibiting effect of sulphur on nitrification (Seitzinger, 1988). In sea water, oxidation of organic carbon proceeds by sulphur reduction which produces sulphide, thus inhibiting the nitrification/denitrification couple. In freshwater, carbon oxidation proceeds by methanogenesis (Seitzinger, 1988).

Iron and molybdenum are trace elements in the nitrate reductase enzyme which suggests that their availability to micro-organisms producing the enzyme may influence denitrification (Keeney, 1973).

The majority of sedimentary  $\text{NO}_3^-$  that is denitrified is generated by nitrification in the sediments (Jenkins and Kemp, 1984).

The factors influencing denitrification are intimately interlinked through coupling between nitrification and denitrification. Nitrate availability in the denitrifying environment depends on the diffusion of nitrate from adjacent aerobic nitrifying environments. This is deemed to be the rate limiting step in denitrification (Andersen *et al.*, 1984). However, the supply of  $\text{NH}_4^+$  limits nitrification so the composition of mineralising organic matter indirectly affects the denitrification rate. Coupling between nitrification and denitrification can also be affected by changes in dissolved  $\text{O}_2$  concentrations.

#### **1.4.6. Dissimilatory nitrate reduction to ammonium**

Reduction of  $\text{NO}_3^-$  to  $\text{NH}_4^+$  without assimilation into organic matter or "Dissimilatory Nitrate Reduction" has also been termed "Nitrate Ammonification" (Binnerup *et al.*,

1992) and "Dissimilatory Ammonium Production (DAP)" (Enoksson and Samuelsson, 1987). This process competes with denitrification for nitrate (Kaspar, 1983). However, Rysgaard *et al.* (1993) found that 100% of  $\text{NO}_3^-$  added to sediments in their experiments was denitrified. They concluded that  $\text{NO}_3^-$  uptake by benthic macrophytes during dark incubations had been misinterpreted as DAP by some previous workers.

Nitrate reduction is a result of bacterial fermentation and respiration (Binnerup *et al.*, 1992; Rysgaard *et al.*, 1993). It occurs under anaerobic conditions in sediments (Buresh and Patrick, 1981). Low redox potential, low nitrate concentrations and high organic matter loading resulting in high organic C: $\text{NO}_3^-$  ratios stimulate  $\text{NO}_3^-$  reduction (Jorgensen, 1989; Binnerup *et al.*, 1992).

Although the capacity of the microbial population to reduce nitrate increases with depth, nitrate reduction was most pronounced in the upper 2cm of Gullmar Fjord sediments, from which it was deduced that nitrate reduction is nitrate limited (Enoksson and Samuelsson, 1987).

Measurement of nitrate reduction has been made indirectly by means of the acetylene block technique (Jorgensen and Sorensen, 1985; Jorgensen, 1989). Acetylene inhibits synthesis of  $\text{N}_2\text{O}$  reductase so that, theoretically,  $\text{NO}_3^-$  added to sediment samples is reduced to  $\text{N}_2\text{O}$  rather than  $\text{N}_2 + \text{N}_2\text{O}$  during denitrification. Dissimilatory nitrate reduction is calculated as the difference between the denitrification from  $\text{N}_2\text{O}$  production and the  $\text{NO}_3^-$  uptake by the sediment. High reported nitrate reduction rates in sediments may be in considerable error due to incomplete inhibition of  $\text{N}_2\text{O}$  reductase by acetylene.

Denitrification rates reported by Enoksson and Samuelsson (1987) using  $^{15}\text{N}$  labelled  $\text{NO}_3^-$  indicate that <3% of nitrate added to estuarine sediments is reduced to  $\text{NH}_4^+$ . Nitrate reduction to ammonia is only possible under rare circumstances such as extreme turbulence which may result in the injection of high  $\text{NO}_3^-$  concentrations into previously  $\text{NO}_3^-$ -free layers containing fermenting or sulphur reducing bacteria (Binnerup *et al.*, 1992). Nitrate injection into deep sediment layers can conceivably also be achieved through nitrate-bearing groundwater being advected through the sediment.

Denitrification rates can be estimated by measuring the decrease in concentration of nitrate in water added to a sediment slurry or core. The estimates of denitrification rates by this method are likely to be too high since nitrate can be assimilated by the biomass and/or possibly reduced to ammonium (Seitzinger, 1988).

## 1.5. Nitrate in groundwater

Restrictions on  $\text{NO}_3^-$  diffusion from the water column to deep sediment layers limit the potential for denitrification at depth (Oremland *et al.*, 1984). Decreasing redox potentials in deeper layers of sediments can result in a greater proportion of  $\text{NO}_3^-$  being reduced to  $\text{NH}_4^+$  (Buresh and Patrick, 1981). This suggests that denitrification or  $\text{NO}_3^-$  reduction to  $\text{NH}_4^+$  can occur in deeper levels of aquatic sediments if nitrate-bearing groundwater enters the sediments from below.

Keeney *et al.* (1971) noted that 36% of nitrogen input to Lake Mendota, Wisconsin enters via the groundwater. As measured from local springs, groundwater contributes mainly  $\text{NO}_3^-$  to Grand Sippewissitt Marsh in such quantities that it largely supports annual plant growth (Valiela *et al.*, 1978).

However, the hydrogeology of an area must be considered before estimating the nutrient contribution of groundwater to an aquatic body. The flow lines of local springs sample water from the upper reaches of a groundwater body, which may be more polluted due to local contaminating sources than deeper groundwater for which the flow lines are much longer and which may originate in uncontaminated areas (Lee, 1980). Evaluating groundwater nutrient input to a water body through hydrogeological cross-sections and quantifying nutrient concentrations from analysis of deep borehole samples avoids these problems, and allowed Loeb and Goldman (1979) to conclude that groundwater contributed 49% of total nitrate-nitrogen input from the Ward Valley catchment area in California to Lake Tahoe.

Extreme nitrate concentrations in aquifers are a relatively recent phenomenon and have been linked to urban development and agriculture (Rawitz *et al.*, 1978; Loeb and Goldman, 1979; Sewell, 1982; Capone and Bautista, 1985; Slater and Capone, 1987; Gonzalez and Romero, 1991; Terao *et al.*, 1993).

Nitrate contributions to aquatic sediments from groundwater have been estimated by correlating heavy rainfall events, which stimulate groundwater flow, with depth increases in interstitial  $\text{NO}_3^-$  concentration and depth decreases in salinity in coastal sediments (Capone and Bautista, 1985). Gonzalez and Romero (1991) correlated heavy rainfall events with near-instantaneous increases in  $\text{NO}_3^-$  concentrations in a shallow, gravel and sand aquifer. Groundwater seepage should occur in any water body with an adjacent landward aquifer, if the water body constitutes a local base level.

Nitrate migration in soils is comparable to the rate of groundwater movement, and nitrate undergoes little or no adsorption in the aquifer (Loeb and Goldman, 1979).

However, denitrification can occur in an aquifer under reducing conditions if there is a suitable denitrifying microbial population. This reduction in groundwater nitrate concentration was found to be limited by nitrate and carbon availability (Slater and Capone, 1987).

## 1.6. Conclusions

The source of nitrogen in living organisms,  $N_2$ , is progressively transformed to organic N,  $NH_4^+$ ,  $NO_2^-$  and  $NO_3^-$  by the processes of fixation, ammonification and nitrification respectively. Denitrification cycles  $NO_3^-$  and  $NO_2^-$  back to  $N_2$  (and  $N_2O$ ). Nitrate reduction to ammonium is quantitatively important only under highly reducing conditions.

In aquatic systems, these microbially-mediated processes occur mostly in the sediments that line aquatic bodies. Nitrogen inputs from riverine and direct sources, and especially from groundwater, can be moderated by denitrification in the sediment lining.

The literature is dominated by descriptions of nitrogen-cycling rates for individual systems and by discussions of the relative merits of measurement methods. However, there is little quantitative information on the effect of the various factors which control the processes of nitrogen cycling.

The significance of the nitrogen cycle in aquatic systems is widely appreciated. However, nitrogen cycling is controlled by site-specific factors which must be investigated on a site-specific basis. The following chapter describes selected features of a specific site: the Verlorenvlei coastal lake.

## Chapter 2 - DESCRIPTION OF THE VERLORENVLEI CATCHMENT AND AIMS OF THE STUDY

### 2.1. Location

The Verlorenvlei coastal lake lies approximately 180km north of Cape Town on the south-west coast of South Africa (Figure 2.1).

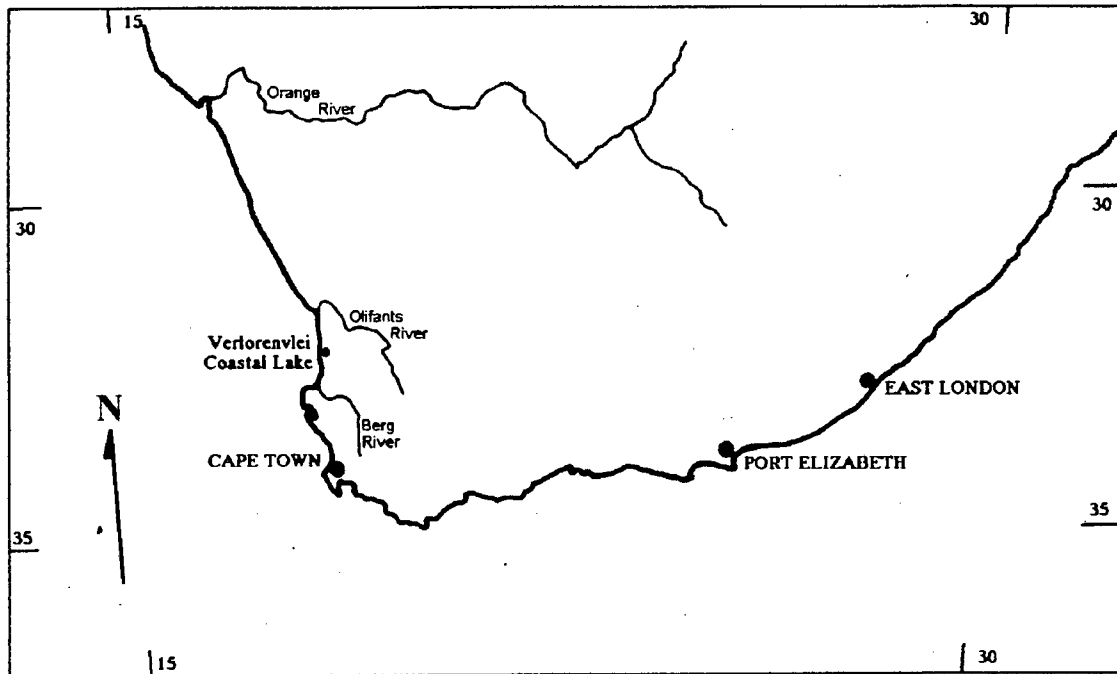


Figure 2.1. Map of the southwestern part of South Africa showing the location of the Verlorenvlei coastal lake. Ticks along the borders of the map are labelled in degrees east and south.

### 2.2. Rainfall and evaporation

The lake and its catchment lie in the winter rainfall area of the Southwestern Cape. The average annual rainfall is approximately 300mm and is extremely variable (Sinclair *et al.*, 1986). The annual evaporation rate of 1140mm far exceeds the rainfall (Sinclair *et al.*, 1986).

The result of the rainfall pattern is that the Verlorenvlei River flows infrequently. The water level in the lake varies considerably throughout the year, the highest level occurring in winter and evaporation causing the level to drop in summer. The drop in water level is accompanied by an increase in water salinity.

### 2.3. Geology

The surface geology of the Verlorenvlei River catchment consists of deposits of the Table Mountain and Malmesbury Groups, and Cenozoic sediments, mostly unconsolidated sands.

The Table Mountain Group sediments outcrop along the southern and north-eastern shores of the lake and consist of white to reddish brown sandstone. The sandstone is medium to coarse-grained, thickly bedded and frequently conglomeratic (Sinclair *et al.*, 1986).

The Malmesbury Group comprises three lithologies: calcareous, quartzose and phyllite with greywacke (Sinclair *et al.*, 1986). These rocks make up approximately 30 per cent of the surface geology of the catchment (Sinclair *et al.*, 1986).

Cenozoic sandy sediments around Elands Bay are derived mostly from unconsolidated barrier and beach deposits with minor components from weathered Table Mountain Group rocks and fluvial deposits from palaeo-river systems (Sinclair *et al.*, 1986). These sediments comprise the primary aquifer for groundwater in the immediate vicinity of the lake (Figure 2.2.).

### 2.4. Ecology

The following description of the ecology of the Verlorenvlei lake is condensed from information in Sinclair *et al.* (1986).

Aquatic vegetation is dominated by the macrophyte *Myriophyllum spicatum* which is rooted in muddy sediments. This plant forms thick weed mats in the lake during the dry season when the water level is low.

There is a great diversity of terrestrial vegetation due to the location of the lake area on the transition between Fynbos and Karroo vegetation types. Fynbos comprises vegetation from the Ericaceae, Restionaceae and Proteaceae families in various proportions depending on altitude and annual precipitation. Karroo vegetation is characterised by a dominance of succulent and drought-resistant varieties and grasses are more abundant. In recent times, anthropogenic influences in the form of crop cultivation and stock grazing have changed the natural vegetation pattern (Meadows *et al.*, 1994).

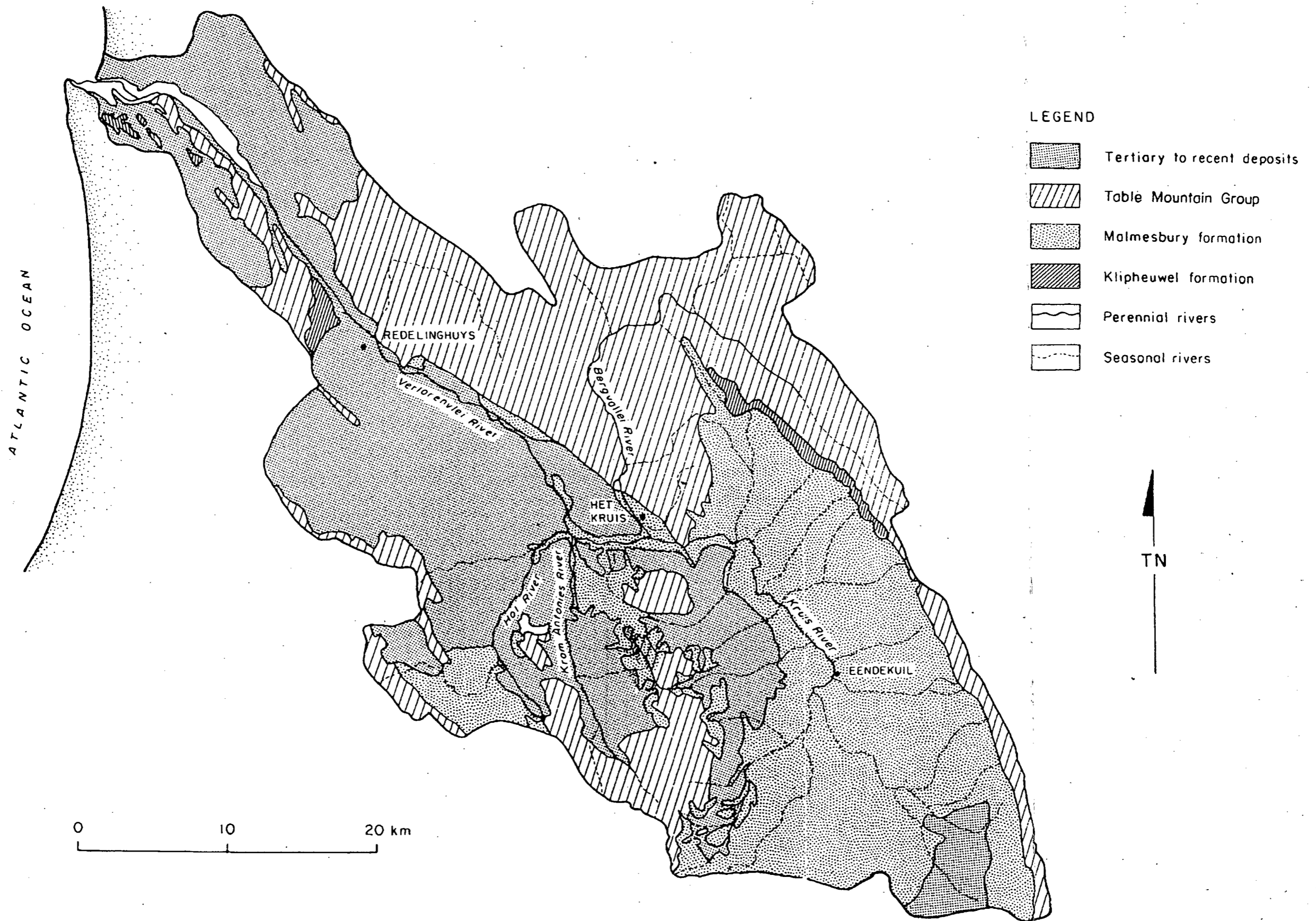


Figure 2.2. Geology of the Verlorenvlei catchment (from Robertson, 1980).

- Is the groundwater polluted with respect to nitrate?
- Do the sediments of the Verlorenvlei Lake have the potential to remove excess nitrate from groundwater?

The aims of this research project were to obtain answers to these questions through the interpretation of data obtained from a programme consisting of sampling of water and sediment, and experiments on sediments. A description of this programme is presented in the following two chapters.

# Chapter 3 - AQUEOUS GEOCHEMISTRY OF THE VERLORENVLEI AND ITS INFLUENT SURROUNDS

## 3.1. Introduction

The previous Chapters have described nitrogen cycling in aquatic sediments and some characteristics of the Verlorenvlei catchment. Since the Verlorenvlei is an important agricultural water resource, its water quality has been studied by Robertson (1980). In this study, a brief section was devoted to discussing the aqueous geochemistry of the lake water.

The aqueous geochemistry of the Verlorenvlei lake is influenced by the river water and groundwater that flow into the lake and interactions between all these waters and the solid phases with which they are in contact. Thus, the geology of the catchment and the intensity and type of land use will determine what ions are removed by runoff and the relative proportions of ions in the catchment waters. When the barrier at the mouth of the lake is breached, the influence of the sea will also affect the geochemistry of the lake waters (Robertson, 1980). However, this event did not occur immediately prior to or during the current study.

Groundwater seepage should occur in any water body with an adjacent aquifer, if the water body constitutes a local base level. The Eland's Bay aquifer lies adjacent to the Verlorenvlei lake. Its characteristics have been investigated during the course of its exploitation as a drinking water resource for the town of Eland's Bay and local farmers (Meyer *et al.*, 1983; Rosewarne, 1986; Jolly, 1992; Maclear, 1994).

This chapter describes the collection of water samples from the lake and from several boreholes along the lake shore. The samples were analysed and the methods of analysis and the data obtained are presented. Interpretation and chemical modelling of the data have led to certain hypotheses regarding the origin and character of the lake water at the time of this study. These hypotheses are elaborated on at the end of the chapter.

## 3.2. Materials and methods

### 3.2.1. Sampling

Water samples were collected from the points indicated on the map of the Verlorenvlei lake area (Figure 3.1). Groundwater samples were collected from existing boreholes and wellpoints and, in one case, from an excavated irrigation dam that fills by seepage from groundwater. Lake water samples were collected from a rowing boat.

No significant temperature or salinity stratification was encountered in a sampling period extending from March to April 1979 (Robertson, 1980). This was ascribed to frequent strong winds which generate waves of sufficient size to ensure complete mixing of the lake water (Robertson, 1980). Similar conditions were encountered during the present study and the assumption was made that stratification of a magnitude sufficient to affect the chemical composition of lake water does not occur in the Verlorenvlei lake. During the present study, the lake depth was measured to be 2.5 metres at the deepest point where samples were taken.

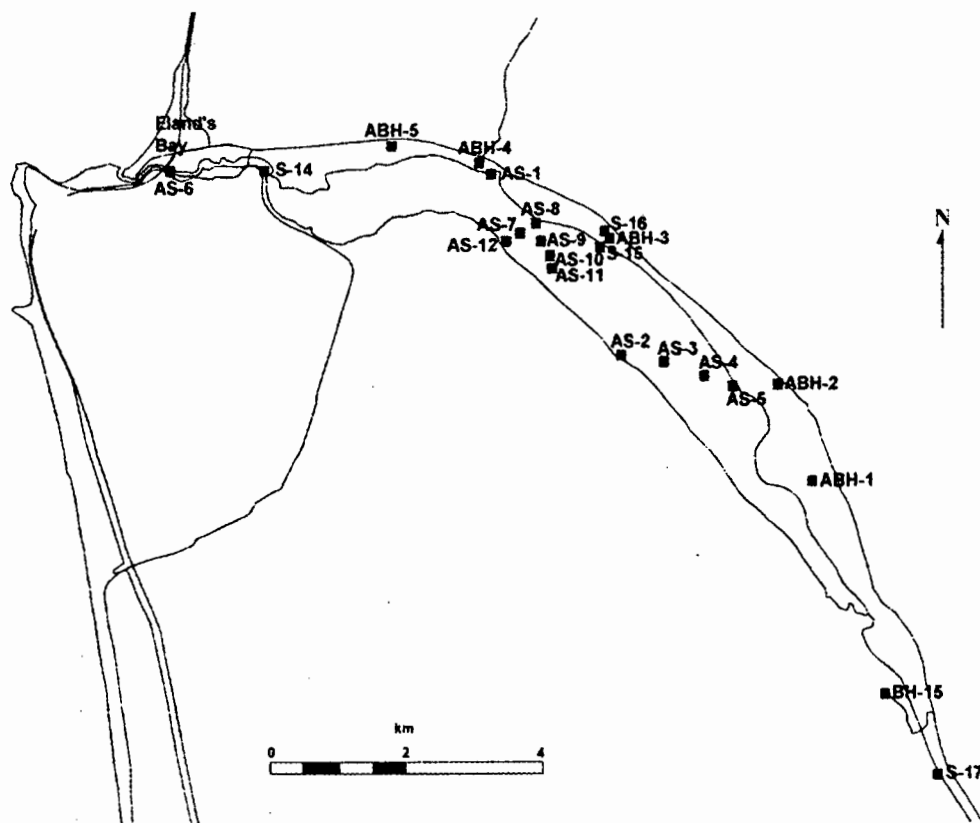


Figure 3.1. Map of the Verlorenvlei lake area showing water sample collection sites.

The positions of sampling points were marked on a 1:50 000 topographic map, in the case of groundwater samples, and 1:10 000 orthophotos, in the case of the lake water samples. Sampling site positions on the lake were determined from three or more compass bearings of landmarks from each site. The site positions were subsequently determined on the orthophoto by triangulation.

Water samples were analysed in the field for electrical conductivity, pH and temperature using a Corning field measurement kit. The equipment was calibrated on site using the standard solutions supplied with the equipment. The standard solutions were checked previously in the laboratory using laboratory standards.

Samples were collected in 500ml clear plastic bottles that were rinsed with the water to be sampled. Two bottles were filled and one of the bottles was treated by the addition of a few drops of concentrated  $\text{HNO}_3$  to bring the pH of the sample down to approximately 2-3 in order to prevent precipitation of cationic species. The filled sample bottles were stored in insulated coolboxes until brought to the laboratory. In the laboratory, the samples were stored at  $\pm 20^\circ\text{C}$  until analysed.

Boreholes and wellpoints sampled were purged using existing installed equipment for 10-15 minutes before collection of the water sample.

### 3.2.2. Analysis

All water samples were analysed by High Pressure Ion Chromatography using a Dionex 3000i ion chromatograph equipped with suitable ion-exchange columns for analysis of major anions and cations. Details of the instrument parameters and eluent concentrations are given in Appendix 1.

Dilution of the samples was necessary to avoid exceeding the ion-exchange capacity of the columns used in the determinations. All samples were diluted by a factor of 50.

### 3.3. Results

Percent charge balance errors (%CBE) for these analyses have been calculated and are given in Tables 3.1 and 3.2. It will be noted that all %CBEs are positive, indicating that the proportion of cations is greater than that of anions in each sample. This is due to the fact that  $\text{HCO}_3^-$  concentrations were not determined on the samples. For this reason,  $\text{HCO}_3^-$  concentrations have been estimated by assuming that the difference between cation and anion species molalities can be attributed entirely to  $\text{HCO}_3^-$ . ( $\text{HCO}_3^-$  concentrations in Tables 3.1 and 3.2 have been shaded to emphasize the origin of the values). This assumption is not strictly correct but is considered to constitute a reasonable approximation in this case since the concentration of  $\text{HCO}_3^-$  is likely to be several orders of magnitude greater than any other anions that have not been determined in the samples. The only other major ion not determined in these samples is  $\text{NH}_4^+$  which is generally present in very low concentrations in natural waters (Hem, 1985). The  $\text{NH}_4^+$  concentration in natural waters analysed in this study is assumed to be negligible.

### 3.3.1. Groundwater

Five boreholes adjacent to the Verlorenvlei lake were sampled. In addition, two samples were taken from an excavated irrigation dam on successive visits. The analyses of these samples for major ions are given in Table 3.1.

Sampling of groundwater in the Verlorenvlei area by the Department of Water Affairs (DWA&F) took place from 24 to 26 March 1993. These analyses have been included in Appendix 2.

Table 3.1. Results of ion chromatography analyses of groundwater samples taken in the vicinity of the Verlorenvlei lake. The  $\text{HCO}_3^-$  concentrations (shaded) have been estimated from the charge balance for each sample. All ion values are in **mg/l** unless otherwise indicated. Nitrate concentrations in bold for clarity.

Sample No.	ABH-1	ABH-2	ABH-3	ABH-4	ABH-5	BH-15	S-16
Locality	Koopmans-drif Farm - spring at edge of vlei	Uithoek Farm - wellpoint	Nuwerus Farm - excavated irrigation dam	Verlorenvlei Country Inn - borehole	Bontheuwei Farm - windpump on borehole	Grootdrif Farm - borehole	Nuwerus Farm - excavated irrigation dam
Sampled	1-Apr-94	1-Apr-94	1-Apr-94	1-Apr-94	1-Apr-94	9-Mar-94	9-Mar-94
EC (dS/m)	29.0	22.3	26.0	28.2	32.8	98.3	100.5
pH	6.18	4.28	6.46	6.25	6.63	-	-
$\text{Cl}^-$	309.0	88.4	196.6	321.0	461.4	295.1	204.0
$\text{NO}_3^-$	<b>21.0</b>	<b>34.1</b>	<b>33.7</b>	<b>27.0</b>	<b>58.8</b>	<b>30.8</b>	<b>34.1</b>
$\text{SO}_4^{2-}$	28.7	27.9	48.5	83.9	125.4	61.2	46.9
$\text{HCO}_3^-$	17.4	0.0	17.8	31.2	0.0	21.3	99.4
$\Sigma_{\text{anions}}$ (mmol <sub>c</sub> /l) excl. $\text{HCO}_3^-$	9.7	3.6	7.1	11.2	16.6	10.1	7.3
$\text{Na}^+$	165.2	54.9	119.7	187.4	268.4	200.1	144.6
$\text{K}^+$	13.9	3.4	4.9	31.6	15.5	16.6	5.5
$\text{Mg}^{2+}$	28.7	14.4	24.7	33.1	39.2	15.5	29.9
$\text{Ca}^{2+}$	0.7	0.2	0.5	1.3	2.1	0.8	0.4
$\Sigma_{\text{cations}}$ (mmol <sub>c</sub> /l)	9.9	3.7	7.4	11.7	15.4	10.4	8.9
% charge balance*	1	1	2	2	4	2	10

\* calculated as:  $\frac{|\Sigma_{\text{cations}} - \Sigma_{\text{anions}}|}{(\Sigma_{\text{cations}} + \Sigma_{\text{anions}})} \times 100\%$

Three of the boreholes sampled by DWA&F were resampled during this study. Comparison of ABH5 with BL2, ABH3/S16 with NS1 and ABH2 with UK1 reveals that the Ca concentration of the groundwater decreased from 1993 to 1994. Samples ABH3/S16 and ABH2 also gained  $\text{SO}_4^{2-}$  over the preceding year. The salinity of ABH2 decreased,

with Na and Cl concentrations lower than in 1993. The concentration of  $\text{NO}_3^-$  was found to be greater than the "maximum limit for no risk" drinking water standard set by the Department of Water Affairs and Forestry (DWA&F) (1993).

### 3.3.2. Lake water

The results of analyses on 14 lake water samples analysed for major ions are given in Table 3.2.

Further lake water analyses from samples collected during 24 to 26 March 1993 were obtained from DWA&F and are included in Appendix 3.

Comparison of the DWA&F analyses with analyses from this study reveals that lake water in April 1994 had less  $\text{Ca}^{2+}$ ,  $\text{Na}^+$  and  $\text{Cl}^-$  than in March 1993. The concentration of  $\text{NO}_3^-$  in lake water was found to be low in both years with two exceptions: AS-6 and AS-11.

Table 3.2. Results of ion chromatography analyses of lake water samples taken from the Verlorenvlei lake. The  $\text{HCO}_3^-$  concentrations (shaded) have been estimated from the charge balance for each sample. All ion values are in **mg/l** unless otherwise indicated. Nitrate concentrations in bold for clarity.

Sample No.	AS-1	AS-2	AS-3	AS-4	AS-5	AS-6	AS-7
Locality Sampled	Lake water 1-Apr-94	Lake water 1-Apr-94	Lake water 1-Apr-94	Lake water 1-Apr-94	Lake water 1-Apr-94	Near mouth 1-Apr-94	Lake water 1-Apr-94
EC (dS/m)	31.1	30.5	30.6	31.4	39.3	404.0	31.5
pH	7.31	8.92	8.78	8.71	8.67	7.77	8.80
Cl	600.8	547.0	555.0	549.8	554.9	7920.3	529.5
<b><math>\text{NO}_3^-</math></b>	<b>2.5</b>	<b>2.5</b>	<b>2.9</b>	<b>2.5</b>	<b>2.5</b>	<b>71.9</b>	<b>2.7</b>
$\text{SO}_4^{2-}$	65.3	75.3	81.2	72.3	75.3	2205.8	79.4
$\text{HCO}_3^-$	109.5	98.8	57.8	95.1	77.3	16569.2	138.1
$\Sigma_{\text{anions}}$ (mmol <sub>c</sub> /l) excl. $\text{HCO}_3^-$	18.3	17.0	17.4	17.0	17.3	270.2	16.7
$\text{Na}^+$	332.8	307.0	299.5	305.8	304.1	10271.0	310.5
$\text{K}^+$	8.7	8.2	8.3	8.0	8.5	300.2	8.9
$\text{Mg}^{2+}$	65.2	61.0	61.0	61.1	60.8	1058.1	62.5
$\text{Ca}^{2+}$	1.4	1.3	1.4	1.4	1.4	5.9	1.4
$\Sigma_{\text{cations}}$ (mmol <sub>c</sub> /l)	20.1	18.6	18.3	18.6	18.5	541.8	18.9
% charge balance*	5	5	3	4	4	33	6

Table 3.2. (continued) Results of ion chromatography analyses of lake water samples taken from the Verlorenvlei lake. The  $\text{HCO}_3^-$  concentrations (shaded) have been estimated from the charge balance for each sample. All ion values are in **mg/l** unless otherwise indicated. Nitrate concentrations in bold for clarity.

Sample No.	AS-8	AS-9	AS-10	AS-11	AS-12	S-14	S-15
Locality Sampled	Lake water 1-Apr-94	Lake water 1-Apr-94	Lake water 1-Apr-94	Lake water 1-Apr-94	Lake water 1-Apr-94	Lake water 9-Mar-94	Lake water 9-Mar-94
EC (dS/m)	31.7	31.0	31.1	30.4	28.7	131.5	102.2
pH	8.85	8.89	8.89	8.84	9.02	-	-
$\text{Cl}^-$	564.0	537.6	532.4	556.0	566.0	1625.2	537.0
$\text{NO}_3^-$	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>24.1</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
$\text{SO}_4^{2-}$	78.8	75.5	76.0	63.7	69.6	104.5	61.6
$\text{HCO}_3^-$	75.3	112.7	106.0	72.3	88.1	305.7	137.8
$\Sigma_{\text{anions}}$ (mmol <sub>c</sub> /l)	17.6	16.8	16.6	17.4	17.4	48.0	16.4
excl. $\text{HCO}_3^-$							
$\text{Na}^+$	309.3	304.6	299.9	307.4	312.0	896.7	306.0
$\text{K}^+$	8.4	8.3	8.3	9.0	8.5	21.6	8.6
$\text{Mg}^{2+}$	61.8	61.8	61.3	59.7	61.2	163.0	62.2
$\text{Ca}^{2+}$	1.3	1.4	1.4	1.4	1.4	1.2	0.8
$\Sigma_{\text{cations}}$ (mmol <sub>c</sub> /l)	18.8	18.6	18.4	18.6	18.9	53.0	18.7
% charge balance*	3	5	5	3	4	5	6

\* calculated as:  $\frac{|\Sigma_{\text{cations}} - \Sigma_{\text{anions}}|}{(\Sigma_{\text{cations}} + \Sigma_{\text{anions}})} \times 100\%$

Sample AS-6 comes from a pool in the channel leading from the lake to the sea. This site is close to the town of Eland's Bay and may be polluted from this source. The high  $\text{NO}_3^-$  concentration in sample AS-11 may be the result of contamination during sampling. Nitric acid was used to acidify cation samples and some acid may have inadvertently been added to the anion sample of AS-11.

### 3.3.3. River water

No samples of river water could be obtained at the time of sampling since the Verlorenvlei River was not flowing. An analysis of river water was obtained from DWA&F. The DWA&F analysis and an analysis of the cation concentrations in river water, from the same site at a different date, obtained by R obertson (1980) are given in Table 3.3.

The analyses indicate that considerable changes in cation concentrations in Verlorenvlei River water occur. Of particular interest is the Ca concentration which decreased by a

factor of  $\pm 3.5$  over the 9.5 year period. The pH of the river water is significantly lower than the lake water (Robertson, 1980).

Table 3.3. Analysis of river water sampled at Redelinghuys 11km upstream of the Verlorenvlei lake. All ion values in **mg/l** unless otherwise indicated. Nitrate concentrations in bold for clarity.

Sample No.	River water (DWA&F)	Robertson (1980)
Sampled	8-May-70	11-Aug-79
EC (dS/m)	18.3	6
pH	8.0	7.2*
Cl	355	
<b>NO<sub>3</sub><sup>-</sup></b>	<b>0.18</b>	
SO <sub>4</sub> <sup>2-</sup>	240	
HCO <sub>3</sub> <sup>-</sup>	40	
$\Sigma_{anions}$ (mmol <sub>c</sub> /l)	15.7	
Na <sup>+</sup>	228	185
K <sup>+</sup>	-	4
Mg <sup>2+</sup>	34	26
Ca <sup>2+</sup>	72	20
$\Sigma_{cations}$ (mmol <sub>c</sub> /l)	16.3	
charge balance*	0.02	
# calculated as:		
$ (\Sigma_{cations} - \Sigma_{anions})/(\Sigma_{cations} + \Sigma_{anions})  \times 100\%$		
* Average of 5 readings taken over the period Nov-75 to Mar-78.		

### 3.3.4. Graphical results

Water analyses and original data were plotted on a Piper diagram. This plot is a method of representing water chemistry according to the relative proportions of major cations and major anions. Six waters from the Verlorenvlei area were plotted onto a Piper plot to ascertain:

1. Possible origins of the waters in terms of mixing between end members.
2. To characterise the type of water in terms of proportions of major anions and cations.

For reasons of clarity it is not useful to plot a very large number of points on a Piper plot. Averages of groups of samples have been plotted in Figure 3.2. The points plotted are:

1. A single sample of river water (obtained from DWA&F) (Table 3.3.).
2. An average of groundwater sample analyses performed by DWA&F (Appendix 2).
3. An average of lake water sample analyses performed DWA&F (Appendix 3).

4. An average of the lake water samples collected and analysed in this study (Table 3.4.).
5. An average of the groundwater samples collected and analysed in this study (Table 3.4.).
6. An average of two analyses of "average" ocean water obtained from the literature: (Henderson, 1982) and (Snoeyink and Jenkins, 1980) (Table 3.4.).

"Star" diagrams (Willis and Hill, 1992) were drawn for the same averages except that the average of DWA&F lake water samples was not plotted. The diagrams are shown in Figure 3.3.

### 3.4. Discussion

Table 3.4. Lake water, groundwater and ocean water average ion concentrations used to plot Piper and Star diagrams in this study.

mg/l	Average ocean water	Average groundwater	Average lake water
Cl <sup>-</sup>	19200.0	278.6	553.9
NO <sub>3</sub> <sup>-</sup>		34.2	2.5
SO <sub>4</sub> <sup>2-</sup>	2705.5	62.6	73.9
HCO <sub>3</sub> <sup>-</sup>	142.0	14.6	93.7
Na <sup>+</sup>	10635.0	166.0	308.4
K <sup>+</sup>	380.0	14.3	8.5
Mg <sup>2+</sup>	1320.0	25.9	61.6
Ca <sup>2+</sup>	406.0	0.9	1.4

#### 3.4.1. Origin of Verlorenvlei water

All of the Verlorenvlei waters plotted in Figure 3.2 are located in Area 7 of the Piper plot. The chemistries of waters in this area of the plot are dominated by alkalis and strong acids. This composition is typical of waters in regions with arid climates (Piper, 1944).

The local groundwater and lake water plot in the same general area on the Piper plot which indicates that they have similar origins. It may be deduced that the groundwater and lake water are in hydraulic continuity. The same conclusion has been derived from the observation that groundwater levels are higher than the water level of the lake which results in groundwater flow towards the lake (Maclear, 1994).

Groundwater and lake water of the Verlorenvlei area are similar in composition to diluted sea water, as indicated by the proximity of the points representing sea water, groundwater and lake water in Figure 3.2. The positions of the points on the plot are not conclusive as regards mixing of compositions of different waters. It is also not clear whether sea water input to the lake in the past has affected the present lake water composition. Discrimination of mixing end members may possibly be achieved using trace elements.

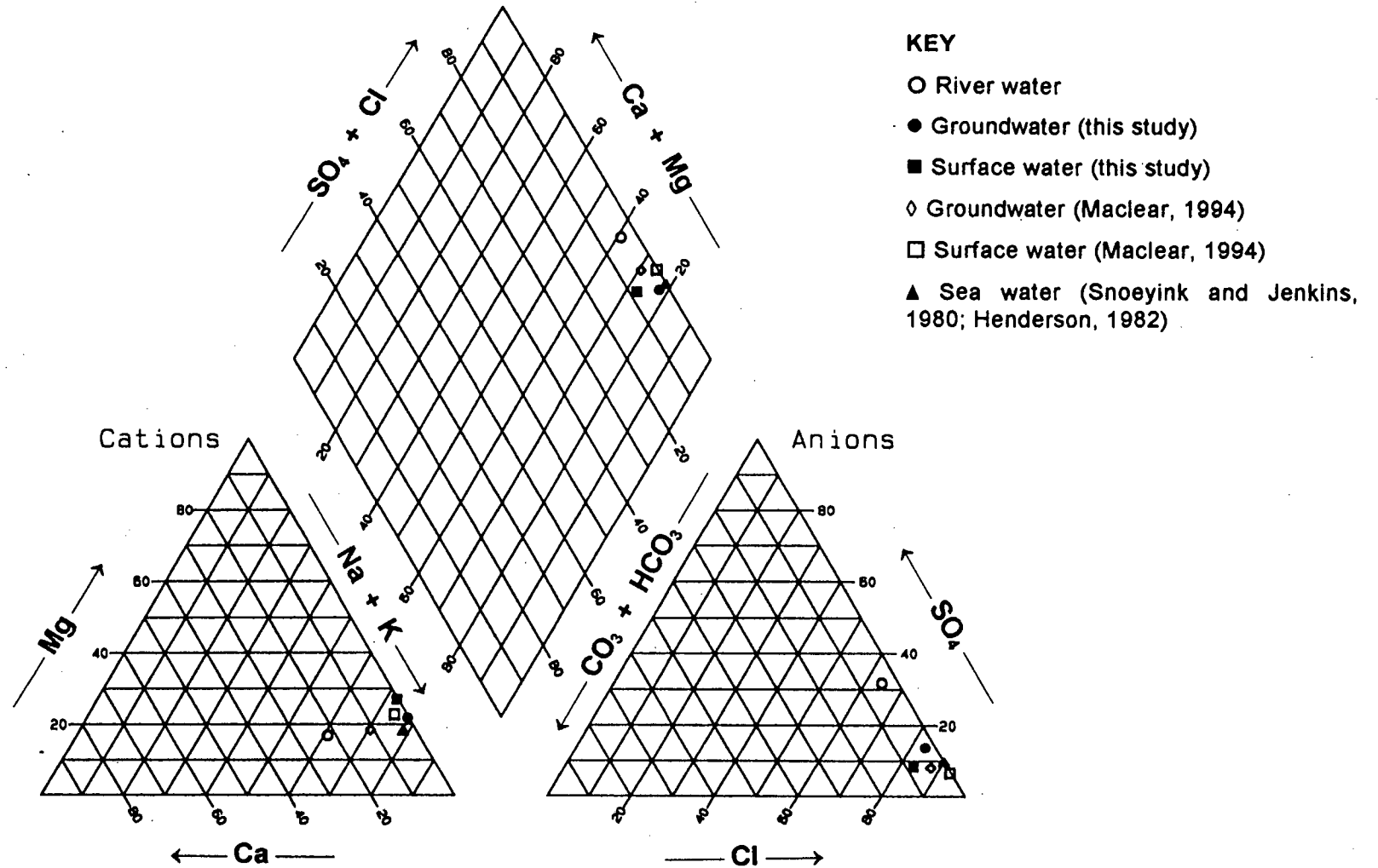


Figure 3.2. Piper plot of six water averages from the Verlorenvlei area.

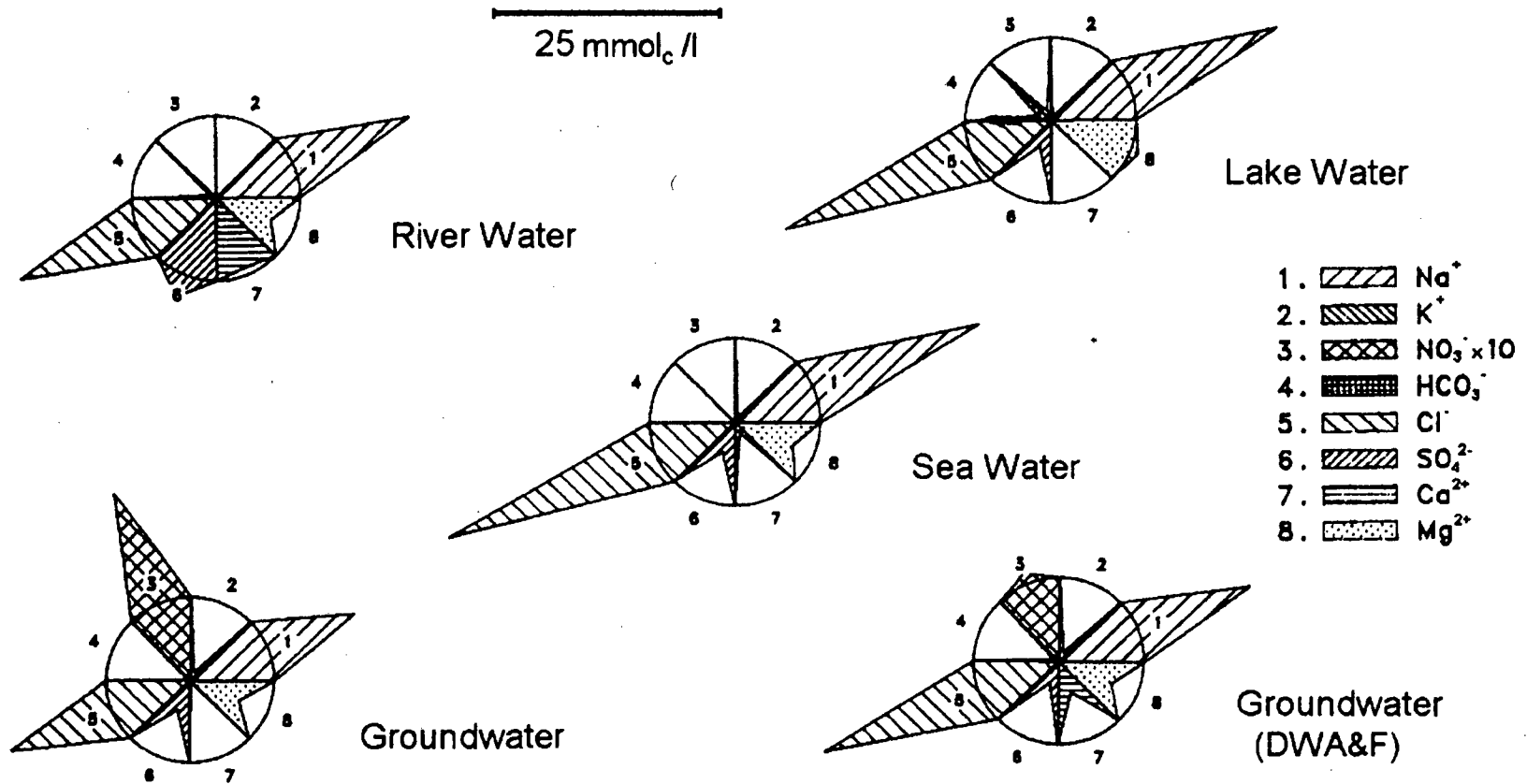


Figure 3.3. Star diagrams (Willis and Hill, 1992) of five water averages from the Verlorenvlei area. "River water" from DWA&F analysis dated 1970, "Lake Water" from surface water sampled in this study, "Sea Water" from literature (see text), "Groundwater" from groundwater sampled in this study, "Groundwater (DWA&F)" from DWA&F analyses dated 1993. The sum of data in each star has been normalised to 100. Length of long axes of segments is proportional to normalised concentration in mmol<sub>c</sub>/l.

River water is enriched in Ca and  $\text{SO}_4^{2-}$  relative to the other waters. In Figure 3.2., a vector from the Ca + Mg 100% and  $\text{SO}_4 + \text{Cl}$  100% apex of the diamond-shaped area through the point representing river water composition passes through the cluster of points representing the averages of lake and groundwater. Since precipitation of a mineral phase drives water composition away from the vertex representing that phase, and river water from higher in the catchment recharges the groundwater and lake water, it may be speculated that gypsum precipitated from the lake water and groundwater of the Verlorenvlei lake area.

This is clearly shown in the star diagrams in Figure 3.3. The river water contains proportionally much more sulphate and calcium than surface lake water or borehole waters. Such a reduction in Ca and  $\text{SO}_4^{2-}$  could be caused by the precipitation of the mineral gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ).

This conclusion, however, is not supported by the solubility value of 2.41g/l for gypsum (Weast, 1975). At this level of saturation, the concentration of Ca would be 560mg/l and that of  $\text{SO}_4^{2-}$  would be 1340mg/l. These concentrations are not attained in lake water in the Verlorenvlei lake since they correspond to a reduction in river water volume through evaporation by over 80% and the actual concentrations in Tables 3.1, 3.2 and 3.3 are very much lower than 560mg/l (Ca) or 1340mg/l ( $\text{SO}_4^{2-}$ ).

It must be assumed that calcium and sulphate are removed from lake water by a different process or that Ca and  $\text{SO}_4$  are not always so high in river water. The DWA river water sample in Table 3.3 was collected in May, which just precedes the rainy season. It is possible therefore that the river water itself is evaporatively concentrated.

Inspection of the concentrations of components in river water and average lake water indicates that a  $\pm 25\%$  reduction in volume through evaporation can account for the increase in salinity of the lake water relative to river water. Speciation calculations (MINTEQA2, 1990) indicated that  $\text{CaCO}_3$  and  $\text{CaMg}(\text{CO}_3)_2$  are likely to precipitate from evaporatively-concentrated river water (Appendix 4). However, biological removal of calcium as shells may be more likely than precipitation.

Sulphur is an important element in cell biochemistry and is assimilated in various forms by certain bacteria (Ehrlich, 1981). Reduction of sulphate to hydrogen sulphide ( $\text{H}_2\text{S}$ ) is an important source of microbial oxygen in anaerobic environments (Ehrlich, 1981). Given the very organic-rich nature of the bottom sediments, it is probable that this is a significant mechanism for the removal of sulphate from Verlorenvlei water.

### 3.4.2. Nitrate-based discrimination of water populations

It may be observed from the analyses that, although other components varied from March 1993 to April 1994, nitrate concentrations in groundwater were noticeably higher than in the lake water. This observation leads to the conclusion that two water populations have been sampled: a groundwater population characterised by high  $\text{NO}_3^-$  concentrations and a lake water population characterised by low  $\text{NO}_3^-$  concentrations.

There were too few analyses of groundwater and lake water from this study to apply a  $\chi^2$  goodness-of-fit test in order to determine whether the populations sampled were normally distributed. For this reason, a non-parametric test was employed to test for equivalence of means.

The Mann-Whitney U test for equivalence of means was applied (STATGRAPHICS, 1992) to the two samples of groundwater analyses and lake water analyses collected in this study, using  $\text{NO}_3^-$  concentration as the discriminating variable. The means were found to be significantly different at the 99.99% confidence level. It was concluded that the groundwater and lake water sampled at Verlorenvlei comprise two separate populations on the basis of their nitrate content.

Tredoux (1993) found the median nitrate concentration of over 18 000 South African borehole water analyses to be 4.5mg/l. Fifteen percent of these analyses had a nitrate concentration in excess of 20mg/l (Tredoux, 1993) which compares with the mean value of 34mg/l obtained for the five groundwaters sampled in this study. The value of 34mg/l exceeds the drinking water "maximum limit for no risk" of 26.6mg/l recommended by the Department of Water Affairs, but is less than the "maximum limit for insignificant risk" of 44mg/l (DWA&F, 1993).

The concentration of nitrate in the groundwater indicates that the groundwater is slightly polluted with respect to drinking water standards. The groundwaters sampled in this study had nitrate concentrations that place them in the top 15% of high-nitrate South African groundwaters.

### 3.5. Conclusions

Verlorenvlei lake water is derived from Verlorenvlei river water that has had Ca and  $\text{SO}_4^{2-}$  removed from it. The removal of calcium ions is achieved through evaporative concentration of river water and the consequent precipitation of  $\text{CaCO}_3$  and  $\text{CaMg}(\text{CO}_3)_2$  or biological removal as shells. Sulphate may be removed through the respiration of sulphate-reducing bacteria in anaerobic sediments.

Comparison of groundwater and lake water analyses from this study with analyses from 1993 indicates that waters from this study have lost Ca. It is possible that this loss is due to precipitation of Ca-carbonate species due to higher evaporation rates in 1994 relative to 1993.

The groundwater adjacent to the lake has a significantly higher concentration of nitrate than the lake water and the groundwater is recharging the lake. The rate of groundwater recharge to the lake is unknown. If the recharge rate is insignificant, then the difference in nitrate concentration may be due to dilution of groundwater with a much greater quantity of lake water. However, if the rate of groundwater recharge is rapid, it may be concluded that excess nitrate is removed from the groundwater as the groundwater flows into the lake.

If the conclusion is correct, then it leads to the hypothesis that the sediment lining of the Verlorenvlei lake may be removing excess nitrate in groundwater flowing through it, thus protecting the lake from possible eutrophication. The capacity of the sediments to remove excess nitrate was examined by conducting experiments on sampled sediments. This is the subject of the next chapter.

## Chapter 4 - DETERMINATION OF SEDIMENT NITRATE-REMOVAL CAPACITY BY EXPERIMENT

### 4.1. Introduction

Previous chapters have been devoted to reviewing the cycling of nitrogen in aquatic sediments, some of the characteristics of the Verlorenvlei aquatic system, and the contrast between elevated nitrate concentrations in groundwater flowing into the lake and significantly lower nitrate concentrations in the lake water. This chapter examines the interaction between the nitrate-rich groundwater and the sediments in the lake as a possible reason for lower nitrate concentrations in the lake water.

In the oceans, nitrogen availability limits phytoplankton growth (Ryther and Dunstan, 1971). By contrast, phosphorus is regarded as limiting in freshwater lakes (Schindler, 1971). Uncontrolled phytoplankton growth, stimulated by nitrogen and/or phosphorus availability, is one of the symptoms of eutrophication. The relationship of nitrogen to phosphorus in this process is not as straightforward as it may seem. Nitrogen also can often be limiting in the freshwater context (Downing and McCauley, 1992).

Downing and McCauley (1992) conclude that total nitrogen to total phosphorus ratios in lakes should be correlated with nutrient sources feeding them. The trophic status of a lake may therefore depend on land use in the catchment which affects the N:P ratio in runoff (Downing and McCauley, 1992). Nutrient cycling in the Verlorenvlei does not seem to have been investigated and there is considerable uncertainty as to the trophic status of the Verlorenvlei lake (Sinclair *et al.*, 1986). Robertson (1980), however, maintains that the lake is oligotrophic.

The fate of groundwater nitrogen in marine sediments has been investigated near Long Island (Capone and Bautista, 1985). It has been speculated that denitrification is the process which determines the fate of groundwater nitrogen in lake sediments (Keeney *et al.*, 1971).

Denitrification rates have been estimated from the addition of  $\text{NO}_3^-$  and/or  $\text{NH}_4^+$  to sediment slurries and sections of sediment cores. This method overestimates denitrification rates because nitrate can be reduced to ammonium or incorporated into organic matter (Seitzinger, 1988). The transformation of  $^{15}\text{N}$ -labelled inorganic compounds added to sediment incubations is a useful and more accurate method of investigating nitrogen-cycling, including denitrification, and has been employed by several authors (Chen *et al.*, 1972; Nishio *et al.*, 1983; Jenkins and Kemp, 1984).

This chapter describes a series of experiments devised to investigate nitrate reduction in Verlorenvlei lake sediments by simulating the interaction between nitrate-rich groundwater and sediment. The method of sample collection and preparation for the experiments is described. Sediment slurries were incubated anaerobically with added  $\text{NO}_3^-$  and the decrease in  $\text{NO}_3^-$  concentration over a 10-day incubation period was measured. The results are presented in tabular form and the significance of the results with reference to the literature previously reviewed is discussed. Certain conclusions that are relevant to nitrate removal in the Verlorenvlei lake have been drawn based on the results.

## 4.2. Materials and methods

Sediment samples were taken from two localities in the Verlorenvlei Lake as indicated on the map in Figure 4.1. The differences in occurrence between the sediments entailed differences in the mode of collection which are described below.

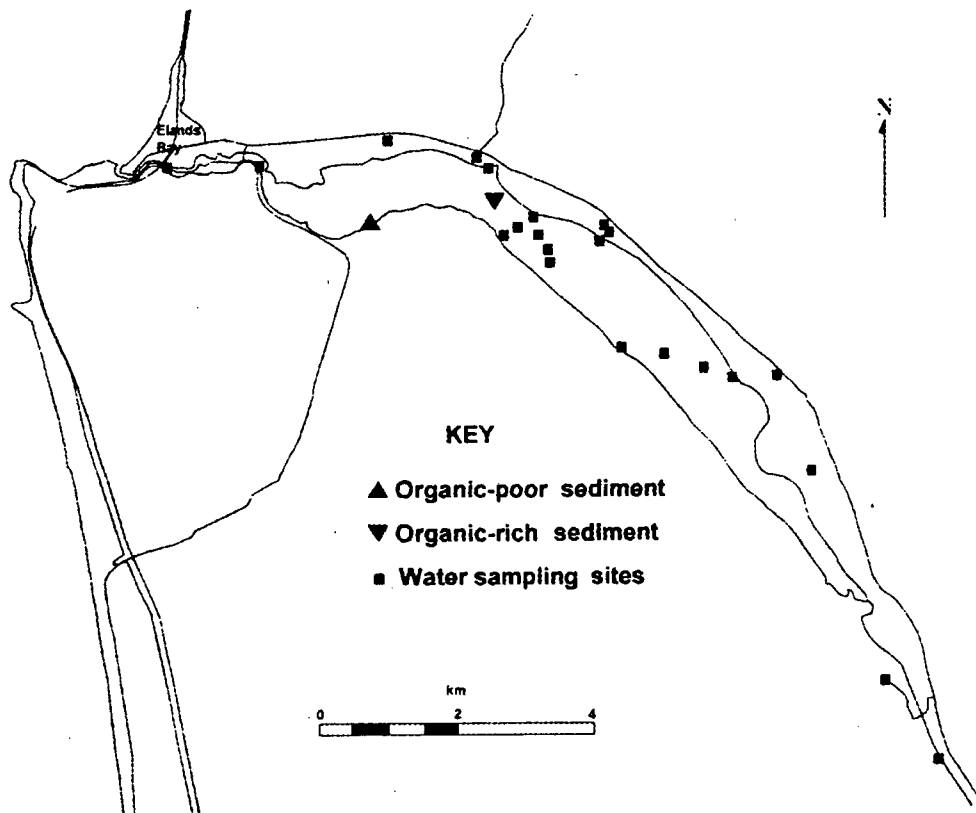


Figure 4.1. Map of Verlorenvlei lake showing sites where sediment samples were collected. The positions of the water sampling sites described in Chapter 3 are included for reference.

### **4.2.1. Sampling**

#### **4.2.1.1. Organic-poor sediment**

This sediment is located along the south-western shores of the lake and was sampled on 2 September 1994. Coring to  $\pm 30$ cm depth at the sampling site indicated that the sedimentary succession is composed of an upper layer of loose, grey sand followed by an intermediate layer of sand cemented by a black pitch-like substance which is underlain by another layer of relatively clean sand. The assumption was made that the black pitch-like substance was refractory organic matter and clay mineral particles on which organic matter had been adsorbed. This layer was sampled in a water depth of  $\pm 50$ cm. An area was cleared of the top layer of clean sand and chunks of the black sand layer were prised out by hand and collected in a ten litre bucket. When full of sand, the bucket was topped up with lake water to minimise the amount of headspace and sealed with a fitted lid which was secured further with adhesive tape. The bucket was transported to the laboratory and stored at  $\pm 20^{\circ}\text{C}$  until used in experiments.

#### **4.2.1.2. Organic-rich sediment**

This sediment is located in the middle of the western portion of the lake and is composed of a visually homogeneous mass of grey-brown mud in which numerous macrophytes are rooted. The transition between water and sediment is gradational and the water content of the sediment decreases slowly with depth resulting in the sediment becoming more viscous and glutinous with depth. The water depth at the sampling site was  $\pm 2$  metres on 8 September 1994. The sample was collected by thrusting a bucket upside down into the sediment until the increase in resistance indicated that a sediment-water mixture, rather than a water-sediment mixture would be collected. The bucket was scooped out of the depression in the sediment and the filled bucket was sealed underwater with a fitted lid. The bucket was further sealed with adhesive tape when it reached the surface. Once transported to the laboratory, the sample was stored at  $\pm 20^{\circ}\text{C}$  until experiments were conducted on it.

### **4.2.2. Sample homogenisation**

For the purposes of conducting repeated multiple experiments, each sediment sample was homogenised. Sediments were transferred under nitrogen atmosphere in a glove box from the ten-litre collection buckets to 10 1-litre wide-mouth, screw-topped jars. The contents of each jar were homogenised by placing the jar in a Turbula mechanical mixer. This device causes the long axis of the jar to precess rapidly, thus mixing the contents thoroughly. Each jar was mixed for  $\pm 20$  minutes.

The contents of each full jar was then transferred under nitrogen atmosphere to a set of 10 clean jars. The contents of each full jar was distributed equally amongst the 10 empty jars until all of the mixed sediment had been apportioned into empty jars. The newly-filled jars were then mixed in the Turbula mixer for  $\pm 15$  minutes. This procedure was repeated once more, resulting in  $\pm 7$  litres of homogenised sediment.

#### **4.2.3. Pre-drying**

Approximately half of each wet, homogenised sediment sample was poured into a plastic-lined cardboard box and left to dry. Dried chunks of organic-poor sediment were hand crushed and sieved through a 2mm sieve to obtain a homogeneous sandy powder for experiment. The dried pieces of organic-rich sediment could not be crushed by hand. Small pieces were loaded into a swing mill and milled for  $\pm 15$  seconds to obtain a fine powder that approximated the original grain size. The powder was used for experiment.

#### **4.2.4. Characterisation of sediment samples**

Each sediment was characterised by determining several variables, as listed below:

1. The water content of the homogenised wet sediment was determined through oven drying at 105°C.
2. The concentration of nitrogen species in the interstitial water of homogenised sediment was obtained through colorimetric analysis for ammonium (section 4.2.6.1.), nitrate and nitrite (section 4.2.6.2.).
3. The percentage of the  $< 53\mu\text{m}$  fraction was determined by wet sieving the wet sediment samples after they had been homogenised.
4. A Walkley-Black organic carbon determination (NSAWC, 1990) was performed on the air-dried sediments (Appendix 5).
5. For the organic-poor sediment, a grain size analysis on the sand fraction was carried out using a settling tube (Appendix 6). A grain size analysis on the organic rich sediment could not be conducted because of an instrument malfunction.
6. The major clay minerals in the organic-rich sediment were identified by X-ray diffraction (Appendix 7). X-ray diffraction on the organic-poor sediment was not indicated because examination of the sand revealed that the mineralogy was dominated by quartz ( $\text{SiO}_2$ ).

#### 4.2.5. Denitrification experiments

##### 4.2.5.1. Sample preparation

The homogenized wet sediment subsamples and the dried subsamples were transferred from their holding containers to 125ml plastic jars under nitrogen atmosphere in a glove box. Nitrogen atmosphere was maintained in order to obtain reducing conditions in the 125ml jars immediately. The jars for continuously wet subsamples were filled to the 100ml mark with sediment. For pre-dried subsamples, 10g of dried sediment was added to each 125ml jar. Groups of three filled jars were then amended with 20ml of a  $\text{NaNO}_3$  solution with concentrations as indicated in Table 4.1.

Six amendments with 3 replicates for a total of 18 jars were prepared for each of the continuously wet sediment and the pre-dried sediment.

Table 4.1. Amendments to sediment subsamples prior to incubation.

Treatment number	N treatment as 20ml $\text{NaNO}_3$ solution		Additional treatment
	(mg/l)	( $\mu\text{mol/l}$ )	
1.	25	0.403	none
2.	25	0.403	glucose (40g/l)
3.	100	1.613	none
4.	100	1.613	glucose (40g/l)
5.	25	0.403	irradiated
6.	25	0.403	glucose (40g/l), irradiated

The amendments marked "irradiated" were subjected to a dose of 500krad of gamma radiation at the Radiobiology Department of Groote Schuur Hospital. It required a total of  $\pm 40$  hours to achieve this dose. The purpose of the radiation treatment was to sterilise the samples.

##### 4.2.5.2. Preparation of amendment solutions

The solutions with which the sediments were amended prior to incubation were made up using Milli-Q™ water that was boiled vigorously for  $\pm 15$  minutes to remove dissolved oxygen. The boiled water was allowed to cool to  $\pm 20^\circ\text{C}$  while  $\text{N}_2$  gas was bubbled through it.

To make up the sodium nitrate solutions, 0.1517g of  $\text{NaNO}_3$  was dissolved in 250ml of the prepared water to obtain a concentration of 100mg N/l as  $\text{NO}_3^-$ . This solution was subsequently diluted to obtain the 25mg N/l as  $\text{NO}_3^-$  solutions. A 10.0g quantity of

analytical grade glucose ( $C_6H_{12}O_6$ ) was added to 250ml of solution to obtain the glucose amendment solutions.

#### 4.2.5.3. Incubation

The 18 jars prepared for each sediment subsample were sealed with adhesive tape and placed in a cardboard box lined with black plastic to exclude light. The boxes and the jars they contained were stored in the laboratory for 10 days. It was intended that the temperature be kept constant by air conditioning but a malfunction during the experiment caused the incubation temperature to vary between 18°C and 26°C.

The incubation period of 10 days was an arbitrary period chosen because no data were available concerning the rate of denitrification in Verlorenvlei sediments. However, an estimate of the residence time of groundwater in the sediment could be made from available geohydrological data.

The permeability of the Verlorenvlei sandy aquifer averages at about 20m/day (Jolly, 1992). An estimate of the velocity of groundwater movement in an aquifer is given by:

$$v = K \frac{H}{L}$$

Where:  $K$  = Hydraulic conductivity (m/day)

$H$  = Hydraulic head (m)

$L$  = Horizontal distance (m)

A hydraulic head of  $\pm 3.5$ m has been reported from the area (Maclear, 1994). The horizontal distance from shore to the sampling site of the organic-rich sediment is approximately 500m. Using the equation and values given above, the groundwater velocity is estimated at 0.14m/day. Thus the 10-day incubation period corresponds to the groundwater traversing a distance of  $\sim 1.4$ m in the sediment.

The experiment described here maintains the same volume of sediment in contact with the same volume of water throughout the incubation period. However, the field situation consists of a given volume of sediment through which fresh volumes of water are moving. The experiment thus measures denitrification potential in the sediments but any extrapolation to the real life situation is impossible given the data obtained.

During the incubations, the minimum and maximum temperature over the previous 24 hours was measured each day using a maximum-minimum thermometer installed in the laboratory. At the time of recording the temperature, each box was opened and each jar

was shaken by hand to encourage dispersion of the liquid throughout the sediment. Then each box was recovered and left for a further 24 hour period.

#### 4.2.5.4. Pore water extraction

At the conclusion of the 10 day incubation, the jars were removed from the boxes and the interstitial liquid was removed from the sediment in each jar by vacuum filtration, centrifugation, or both. Vacuum filtration was performed through filter paper circles using a plastic Büchner funnel attached to a suction pump. Centrifugation was carried out in 50ml plastic centrifuge tubes at 5000rpm for 15-20 minutes.

#### 4.2.6. Analysis of interstitial water by colorimetric methods

##### 4.2.6.1. Analysis for ammonium

The determination of ammonium in the samples was carried out using the phenol-hypochlorite and citrate method to develop indophenol blue as described by Koroleff (1983). This method requires 50ml of sample to which 2ml phenol reagent, 1ml citrate solution and 2ml hypochlorite reagent are added (Koroleff, 1983). The method was modified to the extent that the volumes of the three reagents, phenol, hypochlorite and citrate, added to the sample were scaled down so that 5ml of sample was treated with 0.2ml of each reagent. This required the dilution of the citrate solution by 50% compared to Koroleff (1983). Details of reagent compositions are included in Appendix 8.

The standard method (Koroleff, 1983) recommends heating the sample in order to speed up development of the indophenol blue. Heating is not necessary for colour development (Probyn, pers. comm.) and this was not done on the extracted interstitial water; instead the samples were left overnight and analysed the following day. The  $\pm 12$  hour period has been found to be long enough to fully develop the indophenol blue colour (Probyn, pers. comm.).

Absorbance was measured at 630nm using a Turner Model 690 spectrophotometer. The samples had been prepared in 10cm long borosilicate glass test tubes. Measurement consisted of polishing fingerprints from the test tubes using paper towel squares and inserting the test tube into the spectrophotometer cell. A measurement was made on 2 subsamples, if the absorbance measurements differed by more than 5%, a third subsample was analysed. Readings therefore consisted of the average of two absorbance measurements which differed by less than 5%.

#### 4.2.6.2. Analysis for nitrate and nitrite

Nitrate and nitrite concentrations in the samples were measured simultaneously using a Technicon Autoanalyzer II at the Sea Fisheries Research Institute in Cape Town. The method and manifold layout applied in the determinations was described by Mostert (1983). The method splits the sample between two manifolds and the determination of nitrite and nitrate occur separately but simultaneously. For the determination of nitrite, the sample is reacted first with sulphanilamide and then with n-(naphthyl)-ethylenediamine dihydrochloride to form a red azo dye. The absorbance of the dye is measured at 540nm. For nitrate determination, nitrate in the sample is first reduced to nitrite under alkaline conditions using a column filled with copper-coated cadmium granules. The nitrite is then analysed as described above.

Preparation of the reagents and the copper-cadmium reduction column is described in Appendix 9.

### 4.3. Results

#### 4.3.1. Sediment characteristics

Selected properties of the two sediments are summarised in Table 4.2.

Table 4.2. Sediment characteristics of two sediments from the Verlorenvlei lake.

	Organic-poor sediment	Organic-rich sediment
Locality	Southern shore	Centre of western portion of lake
Description	Quartz sand containing a small quantity of clay and finely-divided black organic matter	Grey-brown mud consisting of illite, kaolinite, muscovite and minor quartz
Grain size (%) <53 $\mu$ m	8	100
>53 $\mu$ m	92	0
Mean grain-size >53 $\mu$ m	270 $\mu$ m	-
Water content of wet sed. (% bymass)	24.3	87.6
<b>Organic carbon content (% by mass)</b>	<b>1.0</b>	<b>11.1</b>

The differences in water content of the wet sediment indicate that a larger mass of organic-poor sediment than organic-rich sediment was used in the continuously wet sediment incubations, even though the volume of sediment plus water was the same in both cases. This fact must be borne in mind when considering the data below.

The inherent concentrations of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$  in the wet, homogenised sediments prior to amendment and incubation are given in Table 4.3.

Table 4.3. Concentrations of nitrate-, nitrite- and ammonium-N in sediments prior to incubation experiments expressed as the mean of three replicates.

Sediment/ Replicate	$\text{NO}_3^-$ -N (mg/l)	$\text{NO}_2^-$ -N (mg/l)	$\text{NH}_4^+$ -N (mg/l)
<b>ORGANIC-POOR SED.</b>			
REP 1	0.31	0.02	4.93
REP 2	0.26	0.03	5.58
REP 3	0.18	0.03	4.85
<b>mean</b>	<b>0.25</b>	<b>0.03</b>	<b>5.12</b>
<b>ORGANIC-RICH SED.</b>			
REP 1	0.25	0.01	23.61
REP 2	0.15	0.00	22.29
REP 3	0.67	0.00	26.33
<b>mean</b>	<b>0.36</b>	<b>0.00</b>	<b>24.08</b>

The maximum and minimum temperatures experienced by the sediment incubations over the course of the incubation period were measured every 24 hours using a maximum-minimum thermometer. A graphical representation of the temperature variation is shown in Figure 4.2.

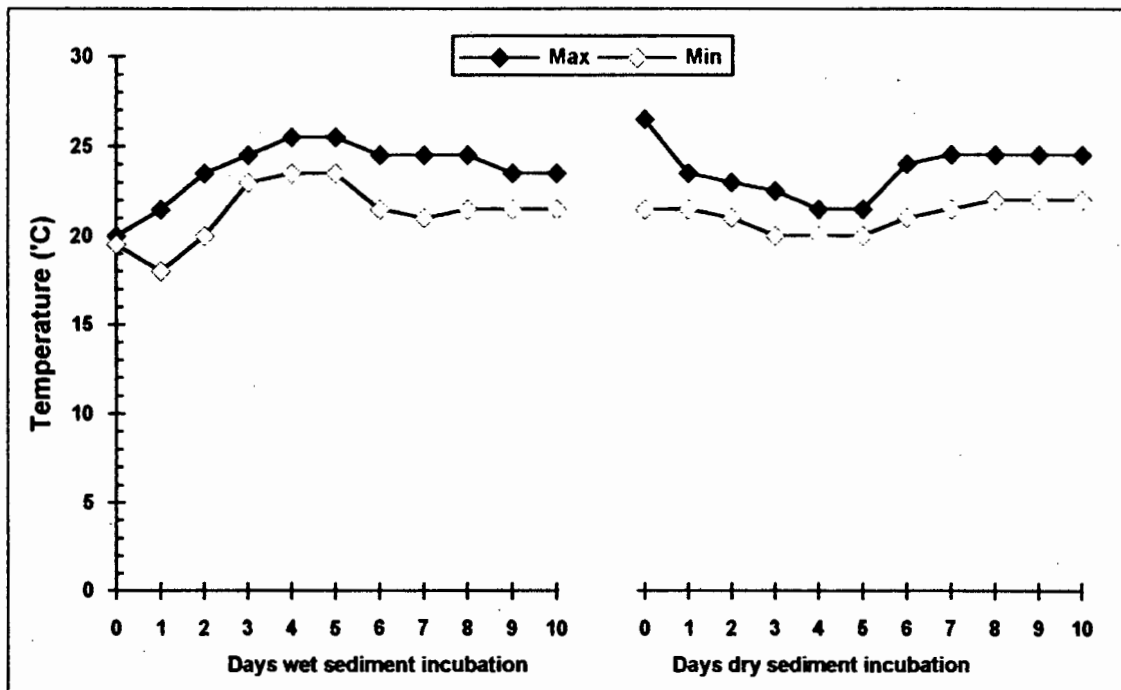


Figure 4.2. Graph of minimum and maximum temperatures over 24 hours experienced by sediment incubations for 10-day incubations of continuously wet and pre-dried sediment.

### 4.3.2. Incubation results

The results of the incubation experiments, expressed as the measured concentrations of nitrate, nitrite and ammonium in pore water are given in Tables 4.4. to 4.7. The range of measured N-species concentrations in samples rather than the standard deviation has been given in Tables 4.4 to 4.7 since it is considered that the standard deviation of only 3 replicates obscures rather than clarifies the spread of the data. The following observations can be summarised from Tables 4.4 to 4.7:

#### 4.3.2.1. Organic-poor sediment

1. The addition of readily oxidizable organic carbon in the form of glucose stimulates the removal of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  from the organic-poor sediment (Tables 4.4 and 4.8).
2. Pre-drying the organic-poor sediment before incubation reduced its capacity to remove smaller quantities of  $\text{NO}_3^-$  since the 25mg/l treatment remained virtually unchanged. In contrast, some  $\text{NO}_3^-$  removal occurred in the 100mg/l treatment (Table 4.4).
3. Nitrite ( $\text{NO}_2^-$ ) is an intermediate in cycling between  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . It has accumulated in the incubated pre-dried organic-poor sediment but not in the incubated continuously wet sediment (Table 4.6).
4. The non-glucose treatments of the pre-dried organic-poor sediment had a lower  $\text{NH}_4^+$  concentration than the same treatment in the continuously wet sediment (Table 4.8).

#### 4.3.2.2. Organic-rich sediment

1. The response of the organic-rich sediment to glucose amendment was not as marked as the organic-poor sediment. In fact, the addition of glucose resulted in slightly more nitrate in the continuously wet glucose amended sediment subsequent to incubation (Table 4.4). However, the low values and high variability in these values imply that differences between values must be regarded with caution.
2. Nitrite ( $\text{NO}_2^-$ ) accumulated to a greater degree in the glucose amendments than in the non-glucose amendments (Table 4.6).

3. In the non-glucose amendments, the ammonium concentration, especially in the pre-dried sediment, increased markedly over the initial value of  $\pm 24\text{mg/l}$  prior to incubation. The  $\text{NH}_4^+$  concentration in the  $100\text{mg/l}$  non-glucose amendments was very similar to the  $\text{NH}_4^+$  concentration in the  $25\text{mg/l}$  non-glucose amendments (Table 4.8).
4. In the glucose amendments, the ammonium concentration decreased compared to the initial level in the sediment, but it decreased to a greater extent in the  $25\text{mg/l}$  amendments than in the  $100\text{mg/l}$  amendments (Table 4.8).

#### 4.3.2.3. General

1. Nitrate removal is more effective in the organic-rich sediment than in the organic-poor sediment (Table 4.4).
2. Although the intention of irradiating the sediments was to sterilise them, this did not occur (Tables 4.5, 4.7, and 4.9).
3. The results from the irradiated sediment incubations mirror what was found in the non-irradiated incubations (Tables 4.5, 4.7, and 4.9). The total incubation period was uncertain, however, since the total 40 hour exposure to radiation was administered during non-continuous periods over 1 week, and the effect of the gamma radiation on the microbe population unknown.

Table 4.4. Nitrate concentrations in interstitial water of two Verlorenvlei sediments, either pre-dried or kept continuously wet, and amended with 25mg/l and 100mg/l nitrate-nitrogen solutions with and without glucose and incubated for 10 days. Mean concentrations have been printed in bold for clarity.

$\text{NO}_3^-$	Organic-poor sediment (initial $\text{NO}_3^-$ -N = 0.25mg/l)				Organic-rich sediment (initial $\text{NO}_3^-$ -N = 0.36mg/l)			
	Glucose		Non-glucose		Glucose		Non-glucose	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
25mg/l $\text{NO}_3^-$ -N CONTINUOUSLY WET	<b>0.02</b>	0.00-0.04	<b>0.18</b>	0.05-0.40	<b>0.60</b>	0.21-0.97	<b>0.08</b>	0.05-0.13
PRE-DRIED	<b>0.12</b>	0.11-0.13	<b>25.91</b>	25.53-26.10	<b>0.18</b>	0.14-0.21	<b>0.19</b>	0.19-0.19
100mg/l $\text{NO}_3^-$ -N CONTINUOUSLY WET	<b>0.09</b>	0.03-0.13	<b>0.25</b>	0.10-0.40	<b>0.26</b>	0.10-0.39	<b>0.17</b>	0.05-0.40
PRE-DRIED	<b>0.31</b>	0.28-0.35	<b>76.96</b>	73.75-80.55	<b>0.05</b>	0.04-0.06	<b>0.19</b>	0.15-0.25

Table 4.5. Nitrate concentrations in interstitial water of two Verlorenvlei sediments, either pre-dried or kept continuously wet, and amended with 25mg/l and 100mg/l nitrate-nitrogen solutions with and without glucose, irradiated with 500krad gamma radiation and incubated for 10 days. Mean concentrations have been printed in bold for clarity.

$\text{NO}_3^-$ (rad)	Organic-poor sediment (initial $\text{NO}_3^-$ -N = 0.25mg/l)				Organic-rich sediment (initial $\text{NO}_3^-$ -N = 0.36mg/l)			
	Glucose		Non-glucose		Glucose		Non-glucose	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
25mg/l $\text{NO}_3^-$ -N CONTINUOUSLY WET	<b>0.11</b>	0.08-0.13	<b>0.23</b>	0.09-0.32	<b>0.32</b>	0.25-0.41	<b>0.21</b>	0.04-0.47
PRE-DRIED	<b>0.00</b>	0.00	<b>21.25</b>	20.67-21.54	<b>1.09</b>	0.32-1.96	<b>0.18</b>	0.17-0.20

Table 4.6. Nitrite concentrations in interstitial water of two Verlorenvlei sediments, either pre-dried or kept continuously wet, and amended with 25mg/l and 100mg/l nitrate-nitrogen solutions with and without glucose and incubated for 10 days. Mean concentrations have been printed in bold for clarity.

$\text{NO}_2^-$	Organic-poor sediment (initial $\text{NO}_2^-$ -N = 0.03mg/l)				Organic-rich sediment (initial $\text{NO}_2^-$ -N = 0.00mg/l)			
	Glucose		Non-glucose		Glucose		Non-glucose	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
25mg/l $\text{NO}_3^-$ -N CONTINUOUSLY WET	<b>0.02</b>	0.01-0.03	<b>0.04</b>	0.03-0.06	<b>0.21</b>	0.01-0.54	<b>0.03</b>	0.02-0.04
PRE-DRIED	<b>0.01</b>	0.00-0.02	<b>4.54</b>	3.40-6.24	<b>0.20</b>	0.15-0.26	<b>0.06</b>	0.06
100mg/l $\text{NO}_3^-$ -N CONTINUOUSLY WET	<b>0.05</b>	0.03-0.07	<b>0.03</b>	0.02-0.03	<b>0.15</b>	0.01-0.29	<b>0.03</b>	0.02-0.04
PRE-DRIED	<b>0.01</b>	0.00-0.01	<b>3.59</b>	2.84-4.54	<b>0.28</b>	0.22-0.34	<b>0.07</b>	0.06-0.07

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Table 4.7. Nitrite concentrations in interstitial water of two Verlorenvlei sediments, either pre-dried or kept continuously wet, and amended with 25mg/l and 100mg/l nitrate-nitrogen solutions with and without glucose, irradiated with 500krad gamma radiation and incubated for 10 days. Mean concentrations have been printed in bold for clarity.

$\text{NO}_2^-$ (rad)	Organic-poor sediment (initial $\text{NO}_2^-$ -N = 0.03mg/l)				Organic-rich sediment (initial $\text{NO}_2^-$ -N = 0.00mg/l)			
	Glucose		Non-glucose		Glucose		Non-glucose	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
25mg/l $\text{NO}_3^-$ -N CONTINUOUSLY WET	<b>0.03</b>	0.03	<b>0.03</b>	0.01-0.03	<b>0.11</b>	0.03-0.21	<b>0.04</b>	0.03-0.07
PRE-DRIED	<b>0.00</b>	0.00-0.01	<b>7.56</b>	7.18-7.75	<b>0.05</b>	0.03-0.08	<b>0.06</b>	0.06-0.07

Table 4.8. Ammonium concentrations in interstitial water of two Verlorenvlei sediments, either pre-dried or kept continuously wet, and amended with 25mg/l and 100mg/l nitrate-nitrogen solutions with and without glucose and incubated for 10 days. Mean concentrations have been printed in bold for clarity.

$\text{NH}_4^+$	Organic-poor sediment (initial $\text{NH}_4^+\text{-N} = 5.12\text{mg/l}$ )				Organic-rich sediment (initial $\text{NH}_4^+\text{-N} = 24.08\text{mg/l}$ )			
	Glucose		Non-glucose		Glucose		Non-glucose	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
25mg/l $\text{NO}_3^- \text{-N}$ CONTINUOUSLY WET	<b>0.27</b>	0.01-0.76	<b>5.73</b>	5.04-6.83	<b>0.10</b>	0.09-0.11	<b>28.30</b>	26.88-29.50
PRE-DRIED	<b>0.00</b>	0.00	<b>3.76</b>	2.91-5.32	<b>0.01</b>	0.00-0.02	<b>103.57</b>	98.25-109.31
100mg/l $\text{NO}_3^- \text{-N}$ CONTINUOUSLY WET	<b>0.05</b>	0.00-0.13	<b>5.55</b>	5.23-6.17	<b>1.87</b>	0.91-2.75	<b>30.50</b>	29.76-31.91
PRE-DRIED	<b>0.00</b>	0.00	<b>3.66</b>	3.16-4.37	<b>1.03</b>	0.08-2.67	<b>97.35</b>	94.78-99.17

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Table 4.9. Ammonium concentrations in interstitial water of two Verlorenvlei sediments, either pre-dried or kept continuously wet, and amended with 25mg/l and 100mg/l nitrate-nitrogen solutions with and without glucose, irradiated with 500krad gamma radiation and incubated for 10 days. Mean concentrations have been printed in bold for clarity.

$\text{NH}_4^+$ (rad)	Organic-poor sediment (initial $\text{NH}_4^+\text{-N} = 5.12\text{mg/l}$ )				Organic-rich sediment (initial $\text{NH}_4^+\text{-N} = 24.08\text{mg/l}$ )			
	Glucose		Non-glucose		Glucose		Non-glucose	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
25mg/l $\text{NO}_3^- \text{-N}$ CONTINUOUSLY WET	<b>0.28</b>	0.00-0.85	<b>5.47</b>	5.31-5.57	<b>3.87</b>	3.15-5.28	<b>35.87</b>	33.97-37.66
PRE-DRIED	<b>0.60</b>	0.00-1.79	<b>3.17</b>	2.95-3.31	<b>25.94</b>	24.93-26.96	<b>107.42</b>	97.61-112.78

## 4.4. Discussion

### 4.4.1. General observations

The primary observation to be made from the results is that, except for dried organic-poor sediment to which no glucose had been added, all of the excess  $\text{NO}_3^-$  added to the amended sediments prior to anaerobic incubation was removed.

Nitrate reduction in sediments and soils under anaerobic conditions can occur along two paths:

Path 1 - Reduction of nitrate to ammonium, or

Path 2 - Denitrification.

Either or both of these reactions occurred in the incubated amended sediments from Verlorenvlei lake. In addition, mineralisation of organically bound N to  $\text{NH}_4^+$  can occur under anaerobic conditions.

Path 1 would result in an increase in the  $\text{NH}_4^+$  concentration of the incubated sediment, and a decrease in the  $\text{NO}_3^-$  and  $\text{NO}_2^-$  concentrations. Addition of glucose stimulates the production of  $\text{NH}_4^+$  from  $\text{NO}_3^-$  (Stanford *et al.*, 1975). Prolonged incubation will result in cell growth and the consequent assimilation (immobilisation) of readily available N to organic N using available carbon sources.

Path 2 would result in a decrease of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  concentrations in the incubated sediment, while  $\text{NH}_4^+$  would remain at its pre-incubation level or increase depending on the extent of mineralisation of organic matter.

The magnitude of both paths is similar (Sorensen, 1978). Sorensen (1978) found that nitrate-reduction to ammonium accounted for 35-42% of added nitrate in a marine sediment incubated under intensely-reducing (-200mV) conditions. In Japanese marine sediments nitrate reduction to  $\text{NH}_4^+$  accounted for 7-52% of added  $\text{NO}_3^-$  (Koike and Hattori, 1978); in freshwater lake sediments it accounted for 40-68% (Chen *et al.*, 1972).

It is unclear which factors determine the path that will be followed, although low redox potential, low nitrate concentrations and high organic C:N ratios in sediments have been cited as favouring nitrate reduction to ammonium (Jorgensen, 1989; Binnerup *et al.*, 1992). Denitrification is stimulated by high nitrate concentrations, comparatively high redox potentials and high organic carbon content (Kaspar, 1983; Oremland *et al.*, 1984; Seitzinger, 1988).

The results of the present sediment incubations are evaluated with this dual nitrate-reduction pathway in mind.

#### 4.4.2. Nitrate reduction to ammonium

The increase of ammonium in the organic-rich sediment after incubation without glucose may be explained by two processes:

1. The ongoing decomposition of organic matter contained in the sediment during incubation, and
2. Dissimilatory reduction of  $\text{NO}_3^-$  to  $\text{NH}_4^+$ .

Since oxidising conditions favour ammonification, the high  $\text{NH}_4^+$  concentrations obtained from the incubation of pre-dried, organic-rich sediment are attributable to the mineralisation of organic matter during pre-drying of the sediment. Less mineralisation occurred during incubation of the continuously wet sediment since this was not exposed to an oxidising environment.

Dissimilatory reduction of  $\text{NO}_3^-$  to  $\text{NH}_4^+$  is accompanied by a decrease in  $\text{NO}_3^-$  and an increase in  $\text{NH}_4^+$  concentration. It is likely that  $\text{NO}_3^-$ -removal in the non-glucose treatments of the organic-rich sediment proceeded by a combination of denitrification and nitrate reduction. However, the data are insufficient to conclude which of the reduction pathways was predominant, or whether both contributed equally to  $\text{NO}_3^-$  removal.

The addition of  $\text{NO}_3^-$  to sediments will poise the redox potential at a level which is more favourable to denitrification than nitrate reduction to ammonium (Buresh and Patrick, 1981). Thus the addition of the  $\text{NO}_3^-$  solution to the incubation may have initially encouraged denitrification of the added nitrate. As nitrate concentrations and redox potential decreased, conditions may have become more favourable for nitrate reduction and a consequent increase in ammonium concentration, especially if the carbon content of the organic matter was low, thus limiting assimilation.

This postulated series of events would explain the observed values of  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and  $\text{NO}_2^-$  in the non-glucose incubations of the organic-rich sediment. The data indicate that a similar set of circumstances prevailed in the continuously wet incubations of the organic-poor sediment.

#### 4.4.3. Immobilisation of ammonium in glucose-amended sediments

The incubation of sediments with glucose resulted in a strong decrease, not only in  $\text{NO}_3^-$  concentrations, but also in  $\text{NH}_4^+$  concentrations in the interstitial water compared to  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations before incubation.

If a source of energy (ie. carbon) is present in excess, heterotrophic bacteria will utilize any available source of nitrogen for incorporation into the cell biomass (Keeney, 1973). This implies that the  $\text{NH}_4^+$  has been consumed by assimilation into the microbial biomass in the glucose-amended sediments.

The relationship between the carbon content of the microbial substrate and  $\text{NH}_4^+$  assimilation has been investigated in coastal sediments from Denmark (Blackburn and Henriksen, 1983). Differences in the rates of ammonium mineralisation and assimilation were found to be due to differences in the composition of organic matter in the sediment. The following equation was used to relate N:C ratios to rates of mineralisation and assimilation:

$$E = \frac{N_s}{N_c} \times \frac{i}{d} \quad (1)$$

Where:  $E$  = efficiency of C incorporation

$N_s$  = N:C in substrate

$N_c$  = N:C in cells

$i$  = rate of  $\text{NH}_4^+$  assimilation

$d$  = rate of  $\text{NH}_4^+$  mineralisation

For any particular group of bacteria,  $E$  and  $N_c$  are constant and (1) becomes:

$$i = \frac{1}{N_s} \times d \times (E \times N_c) \quad (2)$$

As the proportion of C in the substrate increases  $N_s$  becomes smaller and  $i \gg d$ . Net uptake of  $\text{NH}_4^+$  was found to occur when  $N_s < 0.05$  (Blackburn and Henriksen, 1983).

In the present experiment, the addition of glucose ( $N_s = 0$ ) to the amended sediment provided an easily available source of carbon for the microbial population. In the presence of excess carbon, nitrogen became limiting and  $\text{NH}_4^+$  was assimilated to synthesize microbial biomass. The  $\text{NH}_4^+$  assimilation rate became very much greater than the rate of  $\text{NH}_4^+$  mineralisation from organic matter in the sediment with the net result that virtually all available  $\text{NH}_4^+$  was consumed.

It appears that  $\text{NO}_3^-$  denitrification was accompanied by  $\text{NH}_4^+$  assimilation in glucose-amended sediments.

#### 4.4.4. Denitrification

Comparison of the continuously wet sediment incubations of organic-rich and organic-poor sediment with no glucose indicates that  $\text{NO}_3^-$  concentrations decreased while  $\text{NO}_2^-$  remained the same and  $\text{NH}_4^+$  increased slightly. These results would be expected if nitrate reduction occurred by denitrification.

Further, a slightly greater amount of  $\text{NO}_3^-$  remained at the end of the incubation in the organic-poor sediment. Given the low values and high variability among replicates of each treatment, this observation must be regarded with caution. However, the result is expected since denitrification is limited by the organic carbon content of sediments (Seitzinger, 1988).

The Walkley-Black organic carbon contents of stream sediments have been found to be positively correlated with denitrification (Hill and Sanmugadas, 1985). On this basis, the organic-rich sediment would be expected to be a more efficient denitrifier and lower concentrations of nitrate would be expected to remain in the sediment after incubation.

#### 4.4.5. The effect of pre-drying on nitrate removal

Pre-drying did not affect the capacity of the organic-rich sediment to remove excess nitrate. However, pre-drying the organic-poor sediment had the effect of drastically reducing its capacity to remove nitrate. High concentrations of nitrate were accompanied by a slight decrease in initial concentration of  $\text{NH}_4^+$  and a notable increase in  $\text{NO}_2^-$ .

Since  $\text{NO}_2^-$  is an intermediate species in the reduction of  $\text{NO}_3^-$ , its concentration might be expected to increase. However, the rate of nitrite reduction is commonly faster than that of  $\text{NO}_3^-$  with the result that accumulation of  $\text{NO}_2^-$  does not occur. The addition of  $\text{NO}_3^-$  to the dried sediment resulted in conditions that slowed the rate of  $\text{NO}_2^-$  reduction.

The slight decrease in  $\text{NH}_4^+$  concentration may be explained by  $\text{NH}_4^+$  oxidation to nitrate during the pre-drying process.

#### 4.4.6. The effect of gamma radiation

As stated previously, the intention of irradiating the sediments was to sterilise certain treatments. Nevertheless, the experimental results indicated that nitrate transformation occurred in the irradiated treatments. This may have been due to an insufficient radiation dosage (500krad) or the extended time period over which the irradiation took place (40 hours at room temperature). Considerable microbial activity could have occurred during this time.

The option of irradiating the sediments prior to addition of the amendment solutions was rejected. This option would have subjected the jars to additional opening and closing, with the attendant possibility of changing conditions in the irradiated sediments beyond what was intended.

In experiments with Wisconsin lake sediments, Chen *et al.* (1972) subjected sediments to a radiation dosage of 4000krad over an unstated period of time. They observed a 3-fold increase in  $\text{NH}_4\text{-N}$  production compared to non-irradiated sediments and attributed the rapid consumption of added  $\text{NO}_3\text{-N}$  to the release of sufficient enzymes to effect nitrate transformation. It was concluded that irradiation was an unsuitable sterilisation method in N transformation studies (Chen *et al.*, 1972). Unfortunately a copy of this paper could only be obtained after the incubation experiments had already been conducted.

Steam treatment (121°C at 7kg/cm<sup>2</sup> pressure for 30 minutes) slowed down nitrogen transformation rates in autoclaved sediments but did not sterilise the samples (Chen *et al.*, 1972). Azide has been found to stimulate nitrate and nitrite reductase production in several bacterial strains (Payne, 1973).

It seems that the resistance of sediment microbes to radiation and heat precludes the use of sterilised samples in N-cycling investigations.

#### 4.5. Conclusions

The data confirm the hypothesis that the sediments lining the Verlorenvlei lake possess the capability to remove excess nitrate supplied by advection of groundwater through the sediment.

It can be concluded that the sediments have the capacity to remove considerably more nitrate than is currently present in the local groundwater, over a time period that is estimated to be commensurate with the residence time of the groundwater in the sediment. *In situ* organic-poor sediment is seasonally exposed when lake water levels fall. The result of this pre-drying is to eliminate the nitrate removal capacity of the organic-poor sediment. This elimination implies that the organic-rich sediment is responsible for a greater proportion of  $\text{NO}_3^-$  removal since it has a higher content of organic matter and is submerged, and presumably reduced, all year round.

The following conclusions are not fully supported by the experimental data but the results of previous workers (Chen *et al.*, 1972; Stanford *et al.*, 1975; Koike and Hattori,

1978; Sorensen, 1978; Buresh and Patrick, 1981) tend to support the indication that, in the present set of experiments:

1. Addition of  $\text{NO}_3^-$  solutions to the incubated sediments poised the redox potential at a level favourable to denitrification. Nitrate reduction thus initially occurred by denitrification.
2. As  $\text{NO}_3^-$  levels decreased, the redox potential also dropped. Nitrate reduction to ammonium became quantitatively more important as conditions favoured the activities of fermenting and sulphur-reducing bacteria.
3. Where sufficient carbon was present,  $\text{NH}_4^+$  was assimilated into organic forms of nitrogen.

The set of experiments described here may thus be regarded as a reasonable approximation of the situation in which nitrate-contaminated groundwater is introduced into anaerobic Verlorenvlei sediments.

The results of the experiments may therefore be regarded as confirmation that the microbial population resident in Verlorenvlei sediment is capable of reducing excess  $\text{NO}_3^-$ , perhaps by a combination of denitrification and nitrate reduction to ammonium. This nitrate removal capability effectively protects the Verlorenvlei lake from the effects of a high nitrate concentration in groundwater that flows into the lake.

## **Chapter 5 - SUMMARY AND RECOMMENDATIONS**

### **5.1. Summary**

The Verlorenvlei coastal lake lies at the mouth of the Verlorenvlei River on the west coast of South Africa. The river catchment lies in an arid climatic region. Strong seasonal variations in precipitation, and therefore river flow, result in seasonal changes in the water level in the lake and the dissolved content of the lake water.

Water in the lake and its adjacent sandy aquifer are in hydraulic continuity. Groundwater flow is toward the lake. Calcium and sulphate concentrations are lower in groundwater and lake water relative to river water. In the lake water this is probably due to biological and/or physical precipitation of calcium-carbonate minerals and reduction of sulphate to hydrogen sulphide by microbial activity in the lake sediments.

The area surrounding the lake is intensively farmed. Indications are that crop fertilisation under irrigation has resulted in elevated concentrations of nitrate in the groundwater. However, the nitrate concentration of the lake water is significantly lower than in the groundwater. The hypothesis was formulated that nitrate is being removed by microbial activity in the sediments as groundwater flows through them into the lake.

This hypothesis has been tested by experiment through the anaerobic incubation of a sandy, organic-poor sediment and a muddy, organic-rich sediment in the presence of nitrate solutions with differing  $\text{NO}_3^-$  concentrations, some of which were amended with glucose. Results indicated that microbial action does indeed remove nitrate from nitrate solutions added to the sediments. These experimental conditions approximated the situation where nitrate-rich groundwater enters highly-reduced sediments from below. However, after drying the organic-poor sediment lost its capacity to remove nitrate. The addition of easily oxidised organic carbon in the form of glucose was found to stimulate nitrogen removal.

Although the experimental results were not conclusive, they suggested the possibility that nitrate removal occurred by two pathways: denitrification and nitrate reduction to ammonium.

### **5.2. Recommendations**

Some suggestions for future research on this topic are listed below:

- The present study employed slurried sediments. Information on variables such as groundwater flow rate through the sediments and denitrification rates, might be obtained through the use of whole cores. The flow of nitrate-rich groundwater from the bottom to the top of the cores could be easily simulated.
- Although the nitrate removal capacity of the sediments has been confirmed, the measurement of actual rates of removal remains an opportunity for further research.
- Potential for future study exists in determining the relative amount of nitrate that is reduced by each of the denitrification and dissimilatory reduction to ammonium pathways, and the conditions which control these two transformations. Investigations such as these are best conducted using  $^{15}\text{N}$  to isolate the contributions of more than one active process.
- The failure in this and other studies to obtain sterilised samples through gamma irradiation may stimulate future workers to find a more reliable method of sediment sterilisation.
- The geochemistry of the catchment waters and their seasonal variations in composition should be examined in more detail than has been possible in this study or in previous studies.

### 5.3. Conclusions

The three questions posed in the introductory chapter have been largely answered by this research. The questions posed and the answers obtained in this study are stated in brief below.

- What is the geochemistry of the water in the Verlorenvlei lake?

Lake water is brackish and dominated by the ions  $\text{Na}^+$  and  $\text{Cl}^-$ . It is low in  $\text{Ca}^{2+}$  and  $\text{NO}_3^-$ . The concentrations of ions are subject to considerable change due to the variability of dissolved input to the lake and precipitation of ionic species.

- Is the groundwater nitrate concentration elevated in comparison to lake water nitrate concentration?

A significantly higher concentration of  $\text{NO}_3^-$  is found in the groundwater relative to the lake water. However, from a drinking water standpoint, the  $\text{NO}_3^-$  concentration of the groundwater is lower than the maximum recommended limit proposed by the Department of Water Affairs and Forestry (DWA&F, 1993).

- Do the sediments of the Verlorenvlei Lake have the potential to remove excess nitrogen from groundwater?

Experimental work has shown that, under anaerobic conditions, two sediments from different sites in the lake and with different organic matter contents have the capacity to remove nitrate from added solutions containing  $\text{NO}_3^-$ . This simulates the situation where nitrate-rich groundwater enters the sediments from below.

## REFERENCES

- Andersen, T.K., Jensen, M.H., and Sorensen, J. (1984). Diurnal variation of nitrogen cycling in coastal, marine sediments I. Denitrification. *Marine Biology* 83: 171-176.
- Barnes, R.O., Bertine, K.K., and Goldberg, E.D. (1975). N<sub>2</sub>:Ar, nitrification and denitrification in southern California borderland basin sediments. *Limnology and Oceanography* 20: 962-970.
- Binnerup, S.J., Jensen, K., Revsbech, N.P., Jensen, M.H., and Sorensen, J. (1992). Denitrification, dissimilatory reduction of nitrate to ammonium, and nitrification in a bioturbated estuarine sediment as measured with <sup>15</sup>N and microsensor techniques. *Applied and Environmental Microbiology* 58: 303-313.
- Blackburn, T.H. and Henriksen, K. (1983). Nitrogen cycling in different types of sediments from Danish waters. *Limnology and Oceanography* 28: 477-493.
- Buresh, R.J. and Patrick, W.H., Jr. (1981). Nitrate reduction to ammonium and organic nitrogen in an estuarine sediment. *Soil Biology and Biochemistry* 13: 279-283.
- Capone, D.G. and Bautista, M.F. (1985). A groundwater source of nitrate in nearshore marine sediments. *Nature* 313: 214-216.
- Chen, R.L., Keeney, D.R., Graetz, D.A., and Holding, A.J. (1972). Denitrification and nitrate reduction in Wisconsin lake sediments. *Journal of Environmental Quality* 1: 158-162.
- Department of Water Affairs and Forestry (DWA&F). (1993). *South African water quality guidelines, Vol. 1.: Domestic use*. 1st ed., Pretoria: Department of Water Affairs and Forestry.
- Downing, J.A. and McCauley, E. (1992). The nitrogen:phosphorus relationship in lakes. *Limnology and Oceanography* 37: 936-945.
- Ehrlich, H.L. (1981). *Geomicrobiology*. 1st ed., New York: Marcel Dekker, Inc.
- Enoksson, V. and Samuelsson, M. (1987). Nitrification and dissimilatory ammonium production and their effects on nitrogen flux over the sediment-water interface in bioturbated coastal sediments. *Marine Ecology Progress Series* 36: 181-189.
- Forsberg, C. (1989). Importance of sediments in understanding nutrient cyclings in lakes. *Hydrobiologia* 176/177: 263-277.

- 
- Gonzalez, A. and Romero, E. (1991). Fast transmission of nitrates into a detritic aquifer (Lepe, Huelva, Spain). *Water Science and Technology* 24: 211-219.
- Grundmanis, V. and Murray, J.W. (1977). Nitrification and denitrification in marine sediments from Puget Sound. *Limnology and Oceanography* 22: 804-813.
- Henderson, P. (1982). *Inorganic Geochemistry*. 1st ed., Oxford: Pergamon Press.
- Hem, J.D. (1985) *Study and interpretation of the chemical characteristics of natural waters*. (3rd ed.) United States Geological Survey Water Supply Paper 2254, United States Government Printing Office.
- Heydenrych, B. (1993). *Farming practices in the Clanwilliam, Lambert's Bay and Elandsbaai areas*. FCC. Report 93/3.
- Hill, A.R. and Sanmugadas, K. (1985). Denitrification rates in relation to stream sediment characteristics. *Water Research* 19: 1579-1586.
- Howarth, R.W., Marino, R., and Cole, J.J. (1988a). Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. *Limnology and Oceanography* 33: 688-701.
- Howarth, R.W., Marino, R., Lane, J., and Cole, J.J. (1988b). Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. *Limnology and Oceanography* 33: 669-687.
- Jenkins, M.C. and Kemp, W.M. (1984). The coupling of nitrification and denitrification in two estuarine sediments. *Limnology and Oceanography* 29: 609-619.
- Jensen, H.B., Jorgensen, K.S., and Sorensen, J. (1984). Diurnal variation of nitrogen cycling in coastal, marine sediments II. Nitrous oxide emission. *Marine Biology* 83: 177-183.
- Jensen, K., Revsbech, N.P., and Nielsen, L.P. (1993). Microscale distribution of nitrification activity in sediment determined with a shielded microsensor for nitrate. *Applied and Environmental Microbiology* 59: 3287-3296.
- Jolly, J.L. (1992). *The geohydrology of the Eland's Bay primary aquifer*. Department of Water Affairs - Directorate of Geohydrology. Report GH 3581.

- Jorgensen, B.B. and Sorensen, J. (1985). Seasonal cycles of O<sub>2</sub>, NO<sub>3</sub> and SO<sub>4</sub> reduction in estuarine sediments: the significance of a NO<sub>3</sub> reduction maximum in spring. *Marine Ecology Progress Series* 24: 65-74.
- Jorgensen, K.S. (1989). Annual pattern of denitrification and nitrate ammonification in estuarine sediment. *Applied and Environmental Microbiology* 55: 1841-1847.
- Kaspar, H.F. (1983). Denitrification, nitrate reduction to ammonium, and inorganic nitrogen pools in intertidal sediments. *Marine Biology* 74: 133-139.
- Keeney, D.R. (1973). The nitrogen cycle in sediment-water systems. *Journal of Environmental Quality* 2: 15-28.
- Keeney, D.R., Chen, R.L., and Graetz, D.A. (1971). Importance of denitrification and nitrate reduction in sediments to the nitrogen budgets of lakes. *Nature* 233: 66-67.
- Klapwijk, A. and Snodgrass, W.J. (1982). Experimental measurement of sediment nitrification and denitrification in Hamilton Harbour, Canada. *Hydrobiologia* 91: 207-216.
- Koike, I. and Hattori, A. (1978). Denitrification and ammonia formation in anaerobic coastal sediments. *Applied and Environmental Microbiology* 35: 278-282.
- Koroleff, F. (1983). Determination of ammonia. In: *Methods of Seawater Analysis (2nd edition)*, ed. Grasshoff, K., Ehrhardt, M., and Kremling, K. Verlag Chemie, Weinheim.
- Lee, D.R. (1980). Groundwater - solute flux. *Limnology and Oceanography* 25: 183-186.
- Loeb, S.L. and Goldman, C.R. (1979). Water and nutrient transport via groundwater from Ward Valley into Lake Tahoe. *Limnology and Oceanography* 24: 1146-1154.
- Mackin, J.E. and Swider, K.T. (1989). Organic matter decomposition pathways and oxygen consumption in coastal marine sediments. *Journal of Marine Research* 47: 681-716.
- Macleary, L.G.A. (1994). *A groundwater hydrocensus and water quality investigation of the Verlorenvlei primary aquifer - Eland's Bay*. Department of Water Affairs - Directorate of Geohydrology. Report GH 3835.
- Meadows, M.E., Baxter, A.J., and Adams, T. (1994). The late Holocene vegetation history of lowland fynbos, Verlorenvlei, Southwestern Cape Province, South Africa. *Historical Biology* (in press):

---

Meyer, R., Duvenhage, A.W.A., Blume, J., Vallenduuk, J.W., and Huysen, R.M.J. (1983). *Report on a geophysical and geohydrological investigation of the ground water potential between Lambert's Bay and Graafwater and the coastal region between Lambert's Bay and Eland's Bay*. CSIR. Report Kon/Gf/79/1.

MINTEQA2. Metal speciation software. Version A3.00. United States Environmental Protection Agency, 1990.

Mostert, S.A. (1983). Procedures used in South Africa for the automatic photometric determination of micronutrients in seawater. *South African Journal of Marine Science* 1: 189-198.

Nishio, T., Koike, I., and Hattori, A. (1983). Estimates of denitrification and nitrification in coastal and estuarine sediments. *Applied and Environmental Microbiology* 45: 444-450.

Non-affiliated soil advisory working committee (NSAWC). (1990). *Handbook of standard soil testing methods for advisory purposes*. Pretoria: Soil Science Society of South Africa.

Oremland, R.S., Umberger, C., Culbertson, C.W., and Smith, R.L. (1984). Denitrification in San Francisco Bay intertidal sediments. *Applied and Environmental Microbiology* 47: 1106-1112.

Payne, W.J. (1973). Reduction of nitrogenous oxides by microorganisms. *Bacteriological Review* 37: 409-452.

Piper, A.M. (1944). A graphic procedure in the geochemical interpretation of water analyses. *Transactions of the American Geophysical Union* 25: 914-923.

Rawitz, E., Burns, S., Etkin, H., Hardiman, R., Hillel, D., and Terkeltoub, R. (1978). Fate of fertilizer nitrogen in irrigated fields under semi-arid conditions. In: *Soil Nitrogen as Fertilizer or Pollutant - Proceedings and report of a research coordination meeting, Piracicaba, 3-7 July 1978*, International Atomic Energy Agency, 195-235.

Robertson, H.N. (1980). *An assessment of the utility of Verlorenvlei water*. M.Sc thesis, University of Cape Town, unpublished.

Rosewarne, P.N. (1986). *Investigation into the groundwater resources of the Eland's Bay area*. Steffen, Robertson and Kirsten (Kaa) Ing. Report KP4934/4.

- Rysgaard, S., Risgaard-Petersen, N., Nielsen, L.P., and Revsbech, N.P. (1993). Nitrification and denitrification in lake and estuarine sediments measured by the  $^{15}\text{N}$  dilution technique and isotope pairing. *Applied and Environmental Microbiology* 59: 2093-2098.
- Ryther, J.H. and Dunstan, W.M. (1971). Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science* 171: 1008-1013.
- Schindler, D.W. (1971). Carbon, nitrogen, and phosphorus and the eutrophication of freshwater lakes. *Journal of Phytoecology* 7: 321-329.
- Seitzinger, S.P. (1988). Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnology and Oceanography* 33: 702-724.
- Sewell, P.L. (1982). Urban groundwater as a possible nutrient source for an estuarine benthic algal bloom. *Estuarine, Coastal and Shelf Science* 15: 569-576.
- Sinclair, S.A., Lane, S.B., and Grindley, J.R. (1986). *Estuaries of the Cape Part II: Synopses of available information on individual systems. Report No. 32: Verlorenvlei (CW13)*. CSIR. Research Report 431.
- Slater, J.M. and Capone, D.G. (1987). Denitrification in aquifer soil and nearshore marine sediments influenced by groundwater nitrate. *Applied and Environmental Microbiology* 53: 1292-1297.
- Smith, C.J., DeLaune, R.D., and Patrick, W.H., Jr. (1985). Fate of riverine nitrate entering an estuary: 1. Denitrification and nitrogen burial. *Estuaries* 8: 15-21.
- Snoeyink, V.L. and Jenkins, D. (1980). *Water Chemistry*. New York: John Wiley & Sons.
- Sorensen, J. (1978). Capacity for denitrification and reduction of nitrate to ammonia in a coastal marine sediment. *Applied and Environmental Microbiology* 35: 301-305.
- Stanford, G., Legg, J.O., Dzienia, S., and Simpson, E.C., Jr. (1975). Denitrification and associated nitrogen transformations in soils. *Soil Science* 120: 147-152.
- STATGRAPHICS. Statistical graphics system. Version 6.0. Manugistics Inc., 1992.
- Stevenson, F.J. (1972). Nitrogen: element and geochemistry. In: *The Encyclopedia of Geochemistry and Environmental Sciences*, ed. Fairbridge, R.W. 1st ed., Vol. IV A. Van Nostrand Reinhold, New York.

---

Terao, H., Yoshioka, R., and Kato, K. (1993). Groundwater pollution by nitrate originating from fertilizer in Kakamigahara Heights, Central Japan. In: *Selected Papers on Environmental Hydrogeology (from the 29th International Geological Congress (I.G.C.) - Kyoto, Japan, Aug 24 - Sep 3, 1992)*, ed. Sakura, Y. Verlag Heinz Heise.

Tredoux, G. (1993). *A preliminary investigation of the nitrate content of groundwater and limitation of the nitrate input*. Water Research Commission. Report 368/1/93.

Valiela, I., Teal, J.M., Volkman, S., Shafer, D., and Carpenter, E.J. (1978). Nutrient and particulate fluxes in a salt marsh ecosystem: Tidal exchanges and inputs by precipitation and groundwater. *Limnology and Oceanography* 23: 798-812.

Weast, R.C., ed. (1975). *Handbook of Chemistry and Physics*. 56th ed., CRC Press, Cleveland, Ohio.

Willis, J.P. and Hill, D.R.H. (1992). "Star" plots - diagrams with strong visual impact for simultaneously displaying variations in four to nine sample characteristics. In: *Elemental analysis of coal and its by-products*, World Scientific Publications, 75-79.

Yoon, W.B. and Benner, R. (1992). Denitrification and oxygen consumption in sediments of two south Texas estuaries. *Marine Ecology Progress Series* 90: 157-167.

## Appendix 1 - High pressure ion chromatography analysis

Samples were manually diluted by a factor of 50 using Milli-Q™ water. Samples were loaded by hand using a syringe to fill the chromatograph sample loop. Eluent conductivities were suppressed using MicroMembrane™ cation and anion autosuppressors. Chromatograms were collected on a Dionex 3000i ion chromatograph and processed using Dionex API-450 software. The following descriptions have been reproduced from Dionex equipment instruction sheets.

### A1.1. Cations

Cation separation was achieved using a Dionex HPIC-CS5 exchange column under the following conditions:

1. Sample loop volume: 50µl
2. Eluent: 20mM Methyl-sulphonic acid
3. Flow rate: 1.0ml/min
4. Detection: Conductivity detector - peak height
5. Calibration: 5 or 6 standards prepared using analytical grade chemicals and Milli-Q™ water to cover the following ranges (mg/l):

Na	0 - 50
K	0 - 5
Mg	0 - 10
Ca	0 - 50

### A1.2. Anions

Anion separation was achieved using a Dionex HPIC-AS4A-SC ion-exchange column under the following conditions:

1. Sample loop volume: 50µl
2. Eluent: 1.80mM Na<sub>2</sub>CO<sub>3</sub>,  
1.70mM NaHCO<sub>3</sub>
3. Flow rate: 2.0ml/min

4. Detection: Conductivity detector - peak area

5. Calibration: 5 or 6 standards prepared using analytical grade chemicals and Milli-Q™ water to cover the following ranges (mg/l):

Cl	0 - 100
NO <sub>3</sub>	0 - 40
PO <sub>4</sub>	0 - 40
SO <sub>4</sub>	0 - 40

## Appendix 2 - Groundwater analyses from DWA

The following data comprise the results of analyses performed on groundwater samples collected by the Department of Water Affairs from the Verlorenvlei area. Sampling sites are indicated on the map in Figure A2.1. The results are reproduced from Maclear (1994).

Table A2.1. Groundwater analyses reproduced from Maclear (1994). Ion values as mg/l.

	BH3	BH5	BH6	BL1	BL2	GF2	HR1
EC (dS/m)	5.1	10.1	11.5	35.6	21.2	15.5	23.5
pH	7.5	7.9	7.8	7.7	7.9	7.1	6.9
Cl	78	257	285	1127	577	427	535
NO <sub>3</sub>	62.2	11.3	31.6	35.2	62.2	10.8	71.2
SO <sub>4</sub>	30	33	46	140	112	45	68
HCO <sub>3</sub>	11.6	6.7	21.4	23.2	31.7	8.5	150.7
$\Sigma$ anions (mol <sub>c</sub> /l)	4.02	8.22	9.85	35.61	20.11	13.28	20.11
Na	55	152	164	638	319	250	307
K	11.5	3	12	22.3	20.9	8.5	18.4
Mg	9	18	23	73	37	23	61
Ca	23	9	30	41	58	11	91
$\Sigma$ cations (mol <sub>c</sub> /l)	4.58	8.62	10.83	36.37	20.35	13.53	23.39
charge balance*	7	2	5	1	1	1	8

(Table continued overleaf)

	HR2	MK1	R1	R2	UK1	VL1	VL2
EC (dS/m)	25.4	14.9	5.9	20.1	6	45.2	88.3
pH	7.5	7.5	8	8.2	6.9	8.3	8.2
Cl	650	391	70	569	148	1356	2984
NO3	4.8	30.0	30.4	0.2	22.5	44.2	104.6
SO4	31	41	13	48	7	234	370
HCO3	153.1	14.0	74.4	59.2	2.4	136.0	161.7
$\Sigma$ anions (mol <sub>c</sub> /l)	21.54	12.58	3.95	18.00	4.72	46.02	96.10
Na	355	210	44	287	72	743	1521
K	9.1	8.1	2.8	6.8	3.6	17.5	177.8
Mg	60	34	6	51	15	117	230
Ca	74	20	60	39	7	125	229
$\Sigma$ cations (mol <sub>c</sub> /l)	24.31	13.14	5.48	18.80	4.81	48.63	101.07
charge balance*	6	2	16	2	1	3	3

	VL3	VL4	WK1	NS1	SA2	Average
EC (dS/m)	54.4	102.9	11.6	8.8	59.6	29.8
pH	8.3	8.4	8	7.5	8	7.8
Cl	1703	3587	222	211	1864	897
NO3	4.4	153.9	60.6	25.1	0.3	40.3
SO4	185	446	88	27	187	113
HCO3	167.1	224.5	36.6	5.5	100.0	73.1
$\Sigma$ anions (mol <sub>c</sub> /l)	54.64	116.50	9.66	7.00	58.05	29.5
Na	856	1854	154	123	964	477
K	97	288.2	11.6	4.4	21.8	39.2
Mg	121	272	16	19	150	70
Ca	152	255	41	12	10	68
$\Sigma$ cations (mol <sub>c</sub> /l)	57.26	123.14	10.36	7.62	55.32	30.9
charge balance*	2	3	3	4	2	4

\* calculated as  $\frac{|\Sigma_{\text{cations}} - \Sigma_{\text{anions}}|}{(\Sigma_{\text{cations}} + \Sigma_{\text{anions}})} \times 100\%$

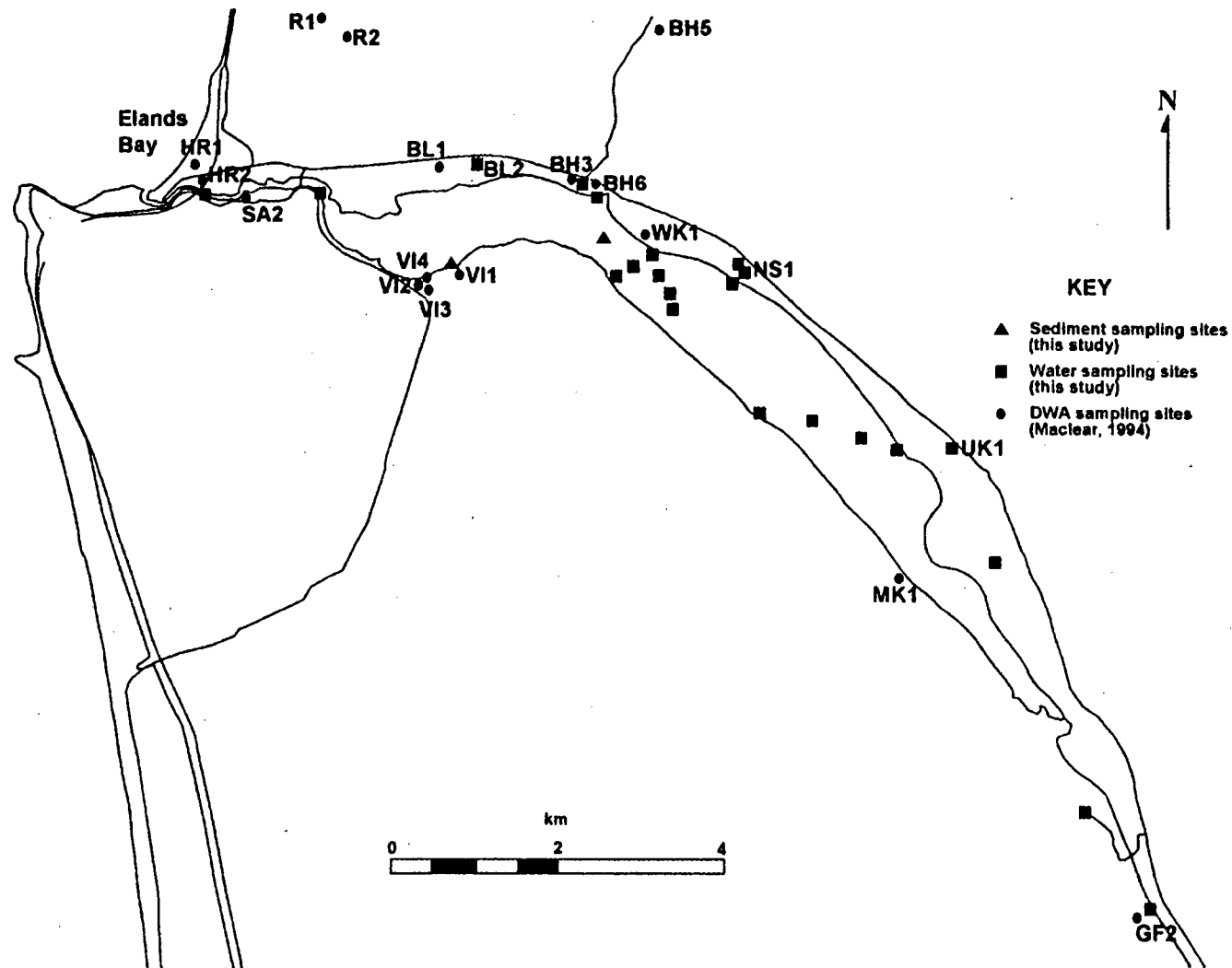


Figure A2.1. Locality map of the Verlorenvlei lake indicating positions of groundwater sampling sites sampled by the Department of Water Affairs. Sediment and water sampling sites from this study have been included on the map. Note that some of the DWA sites and sites from this study are the same.

## Appendix 3 - Lake water analyses from DWA

The following data comprise the results of analyses performed on lake water samples collected by the Department of Water Affairs from the Verlorenvlei area. The results are reproduced from Maclear (1994).

Table A3.1. Lake water analyses reproduced from Maclear (1994). Ion values as mg/l.

Sample No.	VV1	VV3	VV4	VV5	VV7	VV10	Average (excluding VV3)
EC (dS/m)	47.1	683	50.5	37.8	9	34	35.7
pH	7.3	8.1	7.6	7.7	5.4	7.2	7.0
Cl	1555	39305	1473	1152	202	1102	1097
NO3	0.31	0.22	0.22	1.33	9.03	1.28	2.4
SO4	79	5528	112	96	31	24	68
HCO3	119.56	67.71	98.82	81.13	13.42	121.39	86.9
$\Sigma$ anions (mol <sub>c</sub> /l)	47.41	1223.46	45.45	35.80	6.70	33.55	33.8
Na	786	21346	746	589	109	568	560
K	10.3	741.7	12	9.2	6.9	6	9
Mg	129	2456	126	96	17	97	93
Ca	67	819	64	64	19	60	55
$\Sigma$ cations (mol <sub>c</sub> /l)	48.41	1190.39	46.32	36.95	7.26	35.83	35
charge balance*	1	1	1	2	4	3	2

\* calculated as  $|\frac{(\Sigma_{\text{cations}} - \Sigma_{\text{anions}})}{(\Sigma_{\text{cations}} + \Sigma_{\text{anions}})}| \times 100\%$

## Appendix 4 - MINTEQA2 speciation

Reproduced below is selected output from a speciation modelling run using the programme MINTEQA2 and the river water analysis supplied by the Department of Water Affairs. The analysis concentrations have been increased to simulate a reduction in volume of 25% through evaporation. The pH has been changed to 8.5 to approximate the slightly higher pH measured in the lake water during this study.

The results of the speciation model indicate that calcite and dolomite will precipitate from Verlorenvlei River water if 25% evaporation occurs. It is interesting to note that similar modelling runs have indicated that the precipitation occurs even if there is not an increase in pH as the river water flows into the lake.

### A4.1. MINTEQA2 modelling output

PART 1 of OUTPUT FILE

PC MINTEQA2 v3.00 DATE OF CALCULATIONS: 28-NOV-94 TIME: 11:23:26

DWA River water analysis 25% evaporation and pH 8.5

Temperature (Celsius): 25.00

Units of concentration: MG/L

Ionic strength to be computed.

If specified, total carbonate concentration represents total inorganic carbon.

Do not automatically terminate if charge imbalance exceeds 30%

Precipitation is allowed for all solids in the thermodynamic database

The maximum number of iterations is: 40

The method used to compute activity coefficients is: Davies equation

H+	3.187E-06	-8.50
Na+	3.040E+02	-2.00
Mg2+	4.500E+01	-2.85
Ca2+	9.600E+01	-2.75
Cl-	4.730E+02	-2.00
SO4(2-)	3.200E+02	-2.60
HCO3(-)	5.200E+01	-3.19

----- EQUILIBRATED MASS DISTRIBUTION -----							
IDX	NAME	DISSOLVED		SORBED		PRECIPITATED	
		MOL/KG	PERCENT	MOL/KG	PERCENT	MOL/KG	PERCENT
140	CO3-2	3.137E-04	36.2	0.000E-01	0.0	5.540E-04	63.8
500	Na+1	1.324E-02	100.0	0.000E-01	0.0	0.000E-01	0.0
180	Cl-1	1.336E-02	100.0	0.000E-01	0.0	0.000E-01	0.0
732	SO4-2	3.335E-03	100.0	0.000E-01	0.0	0.000E-01	0.0
002	H2O	4.282E-06	100.0	0.000E-01	0.0	0.000E-01	0.0
330	H+1	2.959E-04	100.0	0.000E-01	0.0	0.000E-01	0.0
460	Mg+2	1.725E-03	93.1	0.000E-01	0.0	1.279E-04	6.9
150	Ca+2	1.972E-03	82.2	0.000E-01	0.0	4.261E-04	17.8

CHARGE BALANCE: SPECIATED

SUM OF CATIONS = 1.954E-02      SUM OF ANIONS = 1.927E-02

PERCENT DIFFERENCE = 7.070E-01  $\left[ 100 \times \text{ABS} \frac{(\text{sum anions} - \text{sum cations})}{(\text{sum anions} + \text{sum cations})} \right]$

EQUILIBRIUM IONIC STRENGTH (m) = 2.536E-02

EQUILIBRIUM pH = 8.500

PART 6 of OUTPUT FILE

PC MINTEQA2 v3.00    DATE OF CALCULATIONS: 28-NOV-94    TIME: 11:24: 8

Saturation indices and stoichiometry of all minerals

ID #	NAME	Sat. Index	Stoichiometry( ) of each component
6015000	ANHYDRITE	-1.232	(1.000)150 (1.000)732
5015000	ARAGONITE	-0.139	(1.000)150 (1.000)140
5046000	ARTINITE	-4.217	(-2.000)330 (2.000)460 (1.000)140 ( 5.000) 2
2046000	BRUCITE	-2.883	(1.000)460 (2.000) 2 (-2.000)330
5015001	CALCITE	0.000	(1.000)150 (1.000)140
5015002	DOLOMITE	0.000	(1.000)150 (1.000)460 (2.000)140
6046000	EPSOMITE	-3.781	(1.000)460 (1.000)732 (7.000) 2
6015001	GYPHUM	-1.021	(1.000)150 (1.000)732 (2.000) 2
4150000	HALITE	-5.472	(1.000)500 (1.000)180
5015003	HUNTITE	-4.082	(3.000)460 (1.000)150 (4.000)140
5046001	HYDRMAGNESIT	-11.426	(5.000)460 (4.000)140 (-2.000)330 ( 6.000) 2
5046002	MAGNESITE	-0.496	(1.000)460 (1.000)140
6050001	MIRABILITE	-5.614	(2.000)500 (1.000)732 (10.000) 2
3050000	NATRON	-8.023	(2.000)500 (1.000)140 (10.000) 2
5046003	NESQUEHONITE	-2.905	(1.000)460 (1.000)140 (3.000) 2
6050002	THENARDITE	-6.547	(2.000)500 (1.000)732
5050001	THERMONATR	-9.457	(2.000)500 (1.000)140 (1.000) 2
2015000	LIME	-18.837	(-2.000)330 (1.000)150 (1.000) 2
2015001	PORTLANDITE	-8.716	(-2.000)330 (1.000)150 (2.000) 2
2046001	PERICLASE	-7.601	(-2.000)330 (1.000)460 (1.000) 2

## Appendix 5 - Walkley-Black organic carbon determination

The following description of the method is taken from (NSAWC, 1990). The method utilises a potassium dichromate ( $K_2Cr_2O_7$ ) and sulphuric acid mixture to oxidise organic matter in the sediment sample. Excess dichromate is titrated with iron(II) ammonium sulphate hexahydrate and compared to the titration on a blank. The reduced dichromate is assumed to be equivalent to the organic carbon present in the sample.

### A5.1. Reagents

Potassium dichromate 0.167mol dm<sup>-3</sup>: Dissolve 49.04g potassium dichromate (AR), dried at 105°C, in de-ionised water and make up to 1dm<sup>3</sup> in a volumetric flask.

Sulphuric acid: concentrated, AR grade

Ortho-phosphoric acid: concentrated

Iron(II) ammonium sulphate 0.5mol dm<sup>-3</sup>: Dissolve 196g iron(II) ammonium sulphate hexahydrate in 500cm<sup>3</sup> de-ionised water, add 5cm<sup>3</sup> concentrated H<sub>2</sub>SO<sub>4</sub>, cool and make up to 1dm<sup>3</sup> with de-ionised water

Barium diphenylamine sulphonate indicator: Dissolve 0.4g indicator in 100cm<sup>3</sup> de-ionised water.

### A5.2. Procedure

A measured quantity of sediment was transferred to a 500cm<sup>3</sup> Erlenmeyer flask. A 10cm<sup>3</sup> aliquot of  $K_2Cr_2O_7$  was added to the sample using a pipette and the flask was swirled gently to disperse the sample in the solution. Concentrated sulphuric acid, 20cm<sup>3</sup>, was rapidly added to the solution and the flask was again swirled for approximately 1 minute. The flask was allowed to cool.

When cool, 150cm<sup>3</sup> de-ionised water and 10cm<sup>3</sup> ortho-phosphoric acid were added to the flask. Indicator, 1cm<sup>3</sup>, was added and the contents of the flask were titrated to a green endpoint with iron(II) ammonium sulphate solution.

### A5.3. Results

Table A5.1 shows the results of repeated Walkley-Black organic carbon determinations on the two sediment samples investigated in this study.

Table A5.1. Results of organic carbon determinations on two sediment samples from the Verlorenvlei.

	Replicate	Volume Fe(II) ammonium sulphate added to endpoint	Mass soil in grams	Org C (g kg <sup>-1</sup> )
SED 1 (sand)	1	14.2	0.11	112.1
	2	14.2	0.11	112.1
	3	13	0.11	132.7
	4	14.5	0.11	107.0
			<b>mean org C:</b>	<b>116.0</b>
SED 2 (mud)	1	20.1	0.11	11.1
	2	20.3	0.11	7.7
	3	20.1	0.11	11.1
	4	17.95	0.5	10.5
	5	17.9	0.5	10.7
	6	18	0.5	10.4
			<b>mean org C:</b>	<b>10.0</b>
BLANKS	1	20.6	0	0
	2	20.85	0	0
	3	20.8	0	0

The concentration of  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$  was found to be  $0.483 \text{ mol dm}^{-3}$ .

#### A5.4. Calculation

The carbon content of each determination was calculated using the following equations:

$$\text{organic C\%} = \frac{[\text{cm}^3 \text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \text{ blank} - \text{cm}^3 \text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \text{ sample}] \times M \times 0.3 \times f}{\text{soil mass (g)}}$$

Where  $M$  = concentration of  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$  in  $\text{mol dm}^{-3}$

$f$  = recovery factor of 1.3 to allow for incomplete oxidation of organic matter (NSAWC, 1990)

$$\text{concentration of } \text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \text{ mol dm}^{-3} = \frac{10 \text{ cm}^3 \text{K}_2\text{Cr}_2\text{O}_7 \times 0.167 \times 6}{\text{cm}^3 \text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2}$$

## Appendix 6 - Sand fraction grain size analysis

The grain size of the sand fraction of the organic-poor sediment was determined using a settling tube which consists of a tall ( $\pm 2\text{m}$ ), wide ( $\pm 30\text{cm}$ ) perspex tube filled with water. The sand sample is released at the water surface and settles through the water to the bottom where it falls onto a measuring pan attached to a sensitive balance (Figure A6.1).

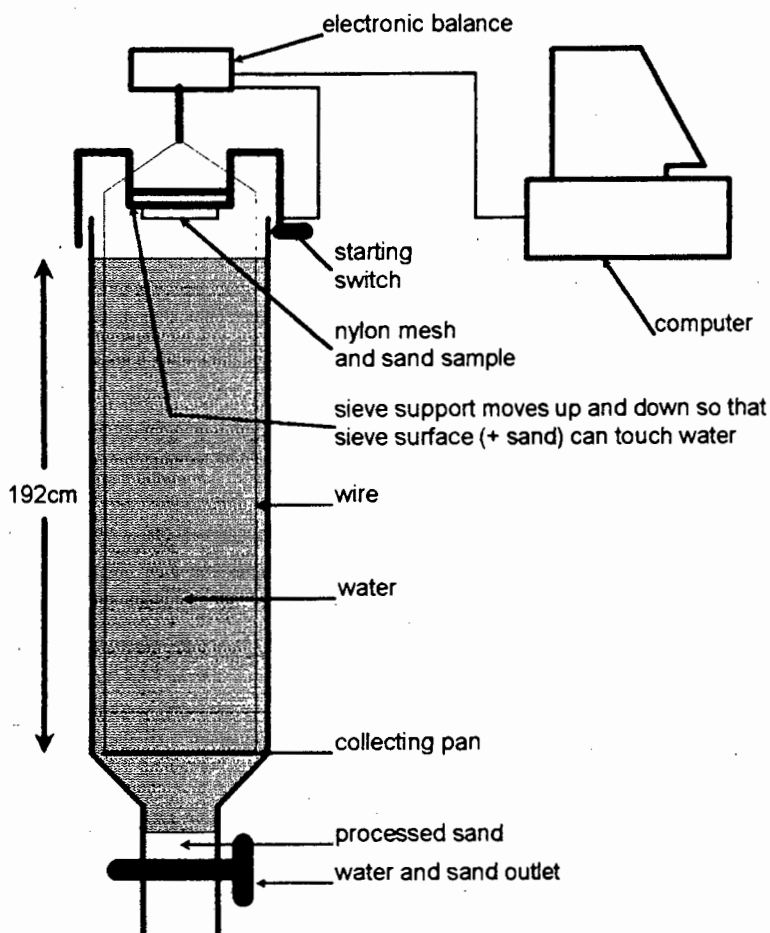


Figure A6.1. Schematic diagram of settling tube apparatus used in the determination of sand size fractions.

Knowing the viscosity of the water in the tube, the density of the grains, the distance the sand grains fall and measuring the time taken for a given mass of the sample to settle to the bottom of the tube, Stoke's law of settling can be applied to determine the size of the grains. This method is particularly accurate when the grains are well-rounded and approach the ideal spherical shape assumed by Stoke's law.

The density of the sand particles was assumed to be 2.65, corresponding to the mineral quartz. From inspection of the sediment, the sand in the organic-poor sediment was found to consist almost entirely of well-rounded quartz grains, making the assumption of

density valid to a first approximation. The grain size of three subsamples of the organic-poor sediment sand fraction were analysed using the settling tube.

Grain size diameters were obtained in  $\phi$  values. Phi values are used preferentially in sedimentological studies in order to facilitate statistical analyses of grain size distributions. Conversion from mm to  $\phi$  values is achieved through the following equation:

$$d = 2^{-\phi}$$

where  $d$  = grain diameter in mm.

### **A6.1. Sample preparation**

The organic-poor sediment was washed in a 53 $\mu$ m sieve so that all particles less than this diameter were washed out of the sand. This included most of the fine, black organic matter in the sediment which was water soluble. No additional removal of organic matter through chemical oxidation was required. A  $\pm 2$ g subsample of the washed sediment was separated. A sample splitter was used so that the grain size distribution in the subsample was not biased in the separation process.

### **A6.2. Analysis**

The subsample was spread over the flat surface of a nylon mesh and dampened using a dilute soap solution to encourage the grains to adhere to the mesh. The mesh and sample was then attached to a magnetic plate suspended over the settling tube so that the surface of the mesh, with the sample adhering to it was facing down the tube Figure A6.1. Analysis commenced by quickly lowering the mesh into the water so that the sand grains were free to settle down the tube. A computer connected to the balance recorded the time since release of the sample and the mass registering on the balance as the sand grains settled. Pertinent data such as water temperature, grain density, settling distance and subsample weight were entered into the computer programme prior to releasing the subsample. Each sample was allowed to settle until the readout indicated a constant mass on the pan before the analysis was terminated. A time of  $\pm 400$  seconds was sufficient to allow all particles down to 0.0625mm in diameter to settle out. A negligible proportion of the grains were found to be smaller than this diameter.

### A6.3. Results

Curves representing the cumulative percentage of each size fraction are shown in Figure A6.2. The statistics derived from each curve by the computer software are shown in Table A6.1.

Table A6.1. Statistics of the population of sand grain sizes obtained from organic-poor sediment sand.

Replicate	Mean $\phi$	Standard deviation	Median $\phi$
1	1.81	0.65	1.85
2	1.90	0.63	1.95
3	1.93	0.71	2.00

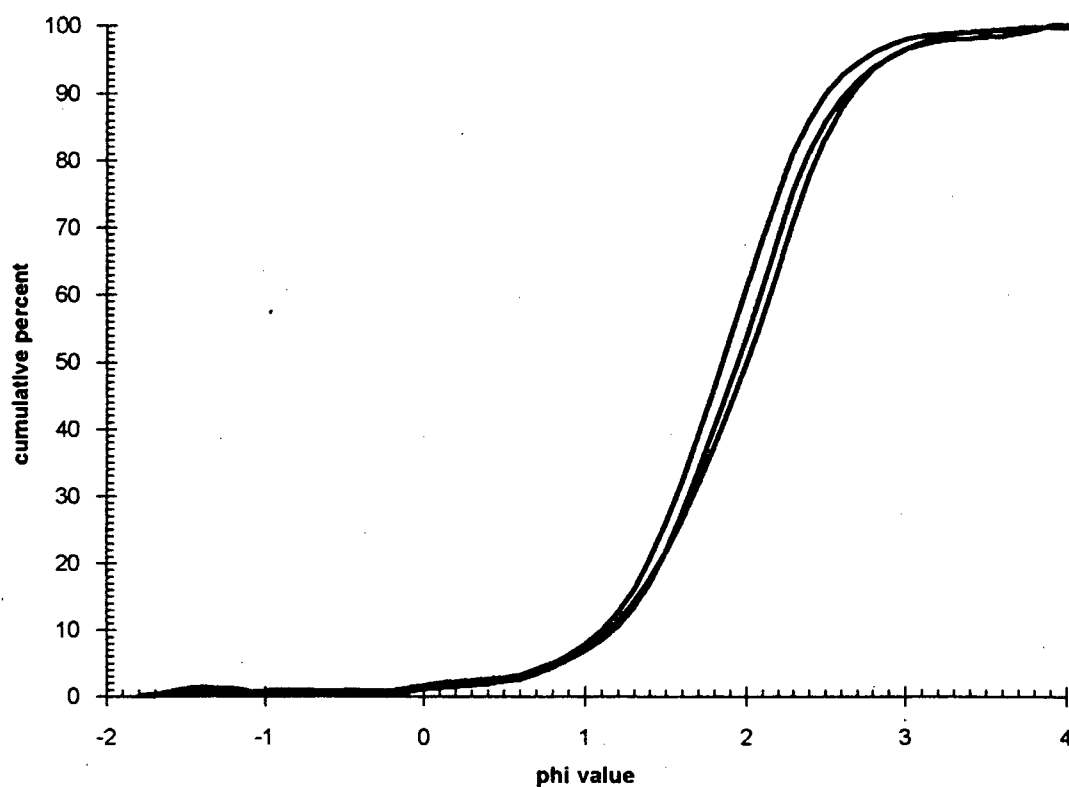


Figure A6.2. Cumulative percent curves for 3 replicates of grain size analysis of the sand fraction of the organic-poor sediment.

## **Appendix 7 - X-ray diffraction analysis**

### **A7.1. Clay Separation**

Only the organic rich sediment was analysed by X-ray diffraction.

A 1M NaOH solution was added dropwise to slurried sediment until the pH of the slurry was stabilised at ~9. The slurry was transferred to a plastic bucket and filled with Na<sub>2</sub>CO<sub>3</sub> solution at pH 10. The suspension was stirred and allowed to stand overnight.

The supernatant clay suspension was tapped off using a plastic hose and glass tube into a separate bucket. The glass tube was inserted to a depth of ~15cm. A 1M HCl solution was added to the clay suspension in dropwise fashion to restore the pH to ~7. Some NaCl was added to promote flocculation of the clay particles. When the clay had flocculated and settled to the bottom of the bucket, the clear supernatant was tapped off and the clay concentrate was transferred to a centrifuge tube. The concentrate comprised the <2µm fraction.

The clay concentrate was centrifuged at ~3000rpm for about 10 minutes. The clear supernatant in the tube was then discarded.

Sodium chloride formed by the neutralisation of NaOH by HCl was washed out of the sample by transferring the clay concentrate to a length of dialysis tubing which was sealed with string at each end and soaked for several days in tap water.

The washed clay concentrate from the sample was resuspended in a bottle by the addition of distilled water. No problems with clay dispersion due to the organic matter content of the sediment were encountered. The concentration of the solution was determined by pipetting a 5ml aliquot of each sample into a porcelain crucible and drying the aliquot to determine the mass of clay per millilitre of suspension.

### **A7.2. X-ray Diffraction**

The clay suspension of the sample was diluted to a concentration of approximately 10mg/ml. Using a glass dropper, a glass slide was coated with the suspension and allowed to dry. The clay particles were thus sedimented onto the glass slide in a preferred orientation.

A Philips PW1050/80 X-ray Diffractometer was used in the clay mineral determination. A copper target X-ray tube was used at a current of 20mA and an excitation voltage of 40kV. The glass slide was inserted into the diffractometer sample chamber and scanned

from  $4-30^\circ 2\theta$ . The diffractograms were analysed using XPLOt software to identify the clay mineral groups present in the sample.

### A7.3. Results

The diffractogram obtained from the analysis of the organic-rich sediment is shown in Figure A7.1. The major peaks have been identified and labelled in the figure.

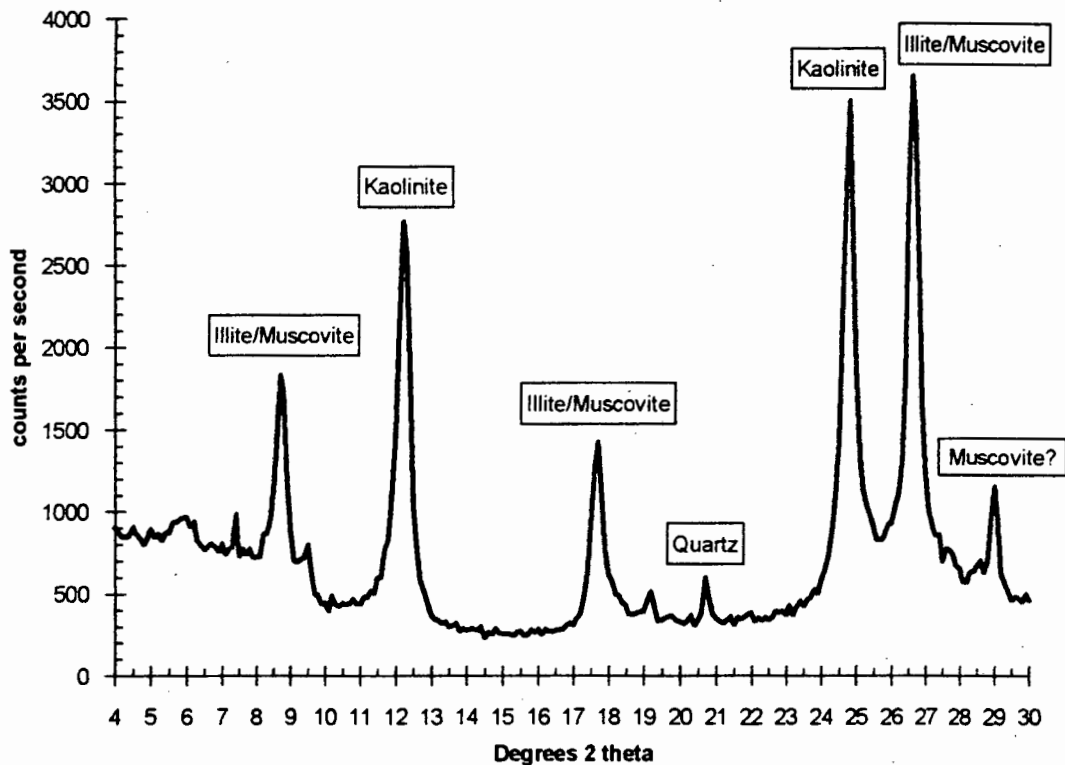


Figure A7.1. Diffractogram obtained from the preferred orientation of clay minerals separated from organic-rich sediment from the Verlorenvlei lake. Unlabelled peaks may be due to the presence of talc.

The diffractogram indicates that the organic-rich sediment is made up of a mixture of kaolinite and illite group clays. Muscovite is also present and a minor component of quartz can also be recognized. No attempt was made to determine definitively or quantitatively the clay minerals by heat treatment or expansion tests.

The minerals present in the sediment are derived from the geology of the Verlorenvlei catchment. Kaolinite and illite are the result of weathering of the Malmesbury Group shales and are also derived from the feldspar and mica components of the Table Mountain Group sandstones (TMS). Muscovite is a mineral commonly found in metamorphic rocks such as the Malmesbury Group and may also be present in the TMS.

The most likely provenance of the quartz in the sediments is the Cenozoic sand formation and TMS outcrops that surround the Verlorenvlei lake.

## Appendix 8 - Ammonium analysis

### A8.1. Reagents

The following reagents are required for the spectrophotometric analysis of ammonium using the phenol and hypochlorite plus citrate method of Koroleff (1983). The following reagent descriptions have been reproduced from Koroleff (1983).

Ammonia-free water should be used to make up all reagents. Commercial ion-exchange resins are suitable for purifying the water. Milli-Q™ water was used for the ammonium determinations in this study.

Sodium hydroxide 1.0mol dm<sup>-3</sup>: Dissolve 40g sodium hydroxide (NaOH) in water and dilute to 1dm<sup>3</sup>.

Phenol reagent: Dissolve 80g of colourless phenol (C<sub>6</sub>H<sub>5</sub>OH) in 300ml ethanol and add 600ml water. Dissolve 600mg disodium nitroprusside dihydrate (Na<sub>2</sub>Fe(CN)<sub>5</sub>NO·2H<sub>2</sub>O) in 100ml water. Add this solution to the phenol solution and store in a refrigerator in a tightly closed amber glass bottle.

Tri-sodium citrate solution: Dissolve 240g tri-sodium citrate dihydrate (C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub>·2H<sub>2</sub>O) in about 500ml distilled water. Make the solution alkaline with 10cm<sup>3</sup> NaOH. Add anti-bumping granules and remove ammonia by boiling until the volume is below 500ml. Cool and dilute to 500ml with water free of NH<sub>3</sub>. Store in a well-stoppered polyethylene bottle. Note: For the determinations in this study, this solution was diluted to 1000ml.

Sodium hydroxide working solution: Add 2ml phenol reagent and 1ml citrate solution to 50ml water. Titrate with the NaOH to a pH of 11.0 using a pH meter. Dilute the 1mol·dm<sup>-3</sup> NaOH so that the pH is 11.0 when 2ml is added to the phenol plus citrate plus water solution. The solution obtained in this way contains about 0.8mol dm<sup>-3</sup> NaOH and is used for preparing the hypochlorite reagent.

Hypochlorite reagent: Dissolve 0.5g Trione, equal to 300mg of available chlorine in 100ml of the working NaOH. Store cold in an amber laboratory glass bottle.

Standard stock solution: Dry ammonium chloride (NH<sub>4</sub>Cl) at 100°C. Dissolve 53.5mg in NH<sub>3</sub>-free water and dilute to 100ml. Preserve with a drop of chloroform. The standard solution contains 10µmol = 140µg·N ml<sup>-1</sup>.

## A8.2. Analysis

A subsample of 5ml was prepared in a 10cm long borosilicate glass culture tube of a diameter to fit the spectrophotometer cell (16mm). Phenol reagent (0.2ml), citrate solution (0.2ml) and hypochlorite reagent (0.2ml) were added with a graduated syringe in such a way that the sample and added solutions were well-mixed. The colour was allowed to develop for 24 hours and the absorbance was measured at 630nm. The colour of the indophenol blue is stable for at least 30 hours (Koroleff, 1983).

A series of standards of concentration 0, 4, 12, 24 and 50  $\mu\text{M}$  were prepared from the standard stock solution. The absorbance was measured and a calibration curve was drawn. The curve is reproduced below. As can be seen from the graph (Figure A8.1), the Lambert-Beer law is valid over the concentration range covered by the standards.

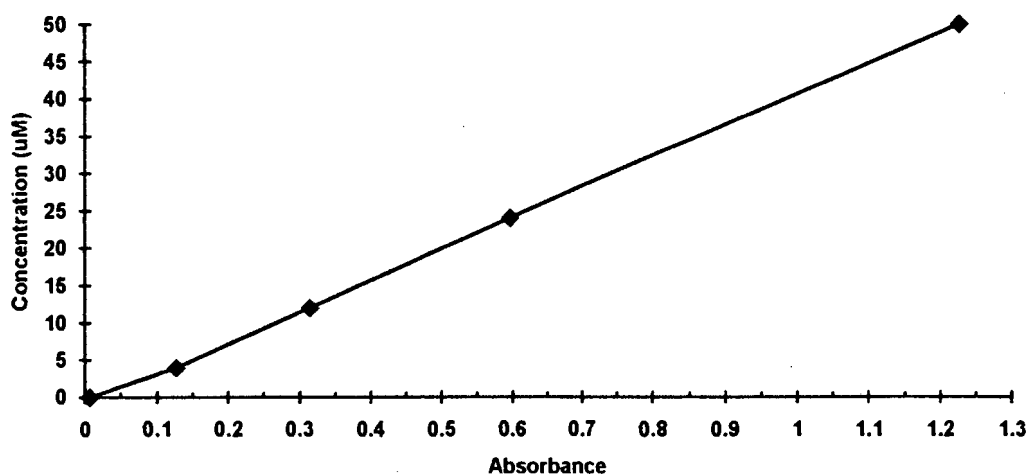


Figure A8.1. Calibration curve obtained for ammonium determinations.

## Appendix 9 - Nitrate and Nitrite analysis

### A9.1. Reagents

The following reagents are required for the automated spectrophotometric analysis of nitrate and nitrite using the method described by Mostert (1983). The following reagent descriptions have been reproduced from Mostert (1983).

#### A9.1.1. Nitrite

Sulphanilamide: 10g of sulphanilamide dissolved in a mixture of 100ml concentrated hydrochloric acid and about 500ml distilled water made up to 1000ml. Store in an amber glass bottle in a refrigerator.

(naphthyl)-ethylenediamine dihydrochloride (Neddi): 0.5g of the amine dissolved in 500ml distilled water and stored in an amber glass bottle in a refrigerator.

Standard nitrite stock solution: 0.345g of dry sodium nitrite dissolved in distilled water and made up to 1 litre. This solution contains 5µg nitrite-N/ml.

#### A9.1.2. Nitrate

Ammonium chloride buffer: 12.5g of ammonium chloride dissolved in 1 litre of distilled water. The pH is adjusted to 8.5 with concentrated ammonia solution and 0.5ml BRIJ-35 is then added per litre of solution.

Sulphanilamide: As for nitrite.

Neddi: As for nitrite.

Reductor filling: Commercially available cadmium granules of 40-60 mesh size.

Copper sulphate solution: 10g of copper sulphate pentahydrate dissolved in 1 litre of distilled water.

Nitrate standard stock solution: 0.5055g of dry potassium nitrate dissolved and made up to 1 litre with distilled water. This stock solution contains 5µg nitrate-N/ml.

Preparation of reductor column: A glass tube of 3mm internal diameter and 120mm total length, drawn out at one end to 1mm diameter is used as the reductor tube. The cadmium granules are stripped of oxides by washing in 50ml of 1M hydrochloric acid, followed by several washes of distilled water to remove the acid. The granules are then

shaken with about 50ml of copper sulphate solution until all the blue colour has been removed. The copperized granules are then gently washed with distilled water until the water is free from colloidal or dispersed copper. The column is then packed with the granules, keeping the granules under water at all times to prevent rapid oxidation.

## A9.2. Analysis

A subsample of 4ml was prepared in a plastic sample cup. For concentrated samples, dilutions were prepared in the cup using calibrated micropipettes. Filled sample cups were loaded onto the autoanalyzer sample loader. A sample:wash ratio of 1:2 was used and controlled by means of a rotating cam in the sample loader.

Standards of 5, 10 and 15 $\mu\text{g NO}_2^-$ -N were prepared in advance of analysis and run at regular intervals among the samples. Standards of 5, 10, 15 and 30 $\mu\text{g NO}_3^-$ -N were also prepared and run in a similar fashion.

A chart recorder was used to record the absorbances of the samples relative to the standards. An example of the output is shown in Figure A9.3.

## A9.3. Autoanalyzer manifolds

### A9.3.1. Nitrite

The layout of the analyzer manifold for nitrite determination is shown in Figure A9.1.

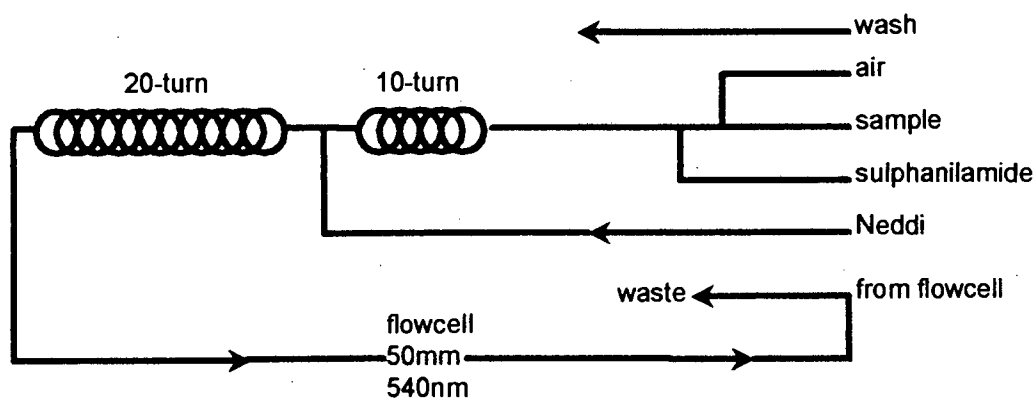


Figure A9.1. Schematic diagram of autoanalyzer manifold layout for the analysis of nitrite in aqueous samples.

## A9.3.2. Nitrate

The layout of the analyzer manifold for nitrate determination is shown in Figure A9.1.

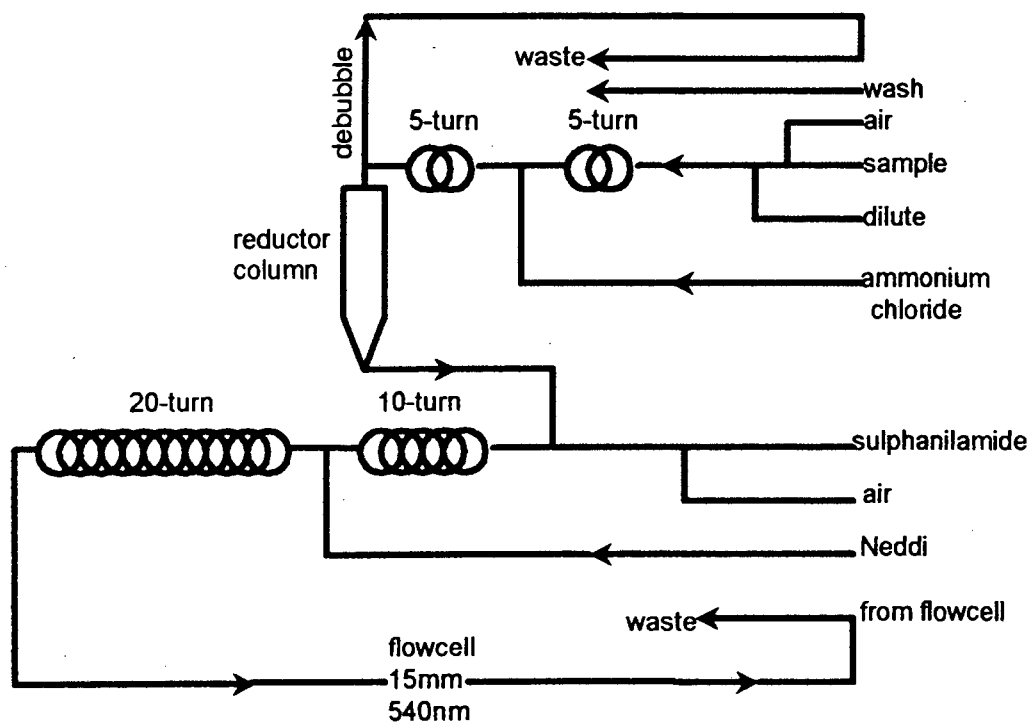


Figure A9.2. Schematic diagram of autoanalyzer manifold layout for the determination of nitrate in aqueous samples.

OVERLEAF:

Figure A9.3. Example of chart recorder output of relative absorbances of samples being analysed for nitrite (red) and nitrate (green). Note that the nitrite and nitrate peaks for the same sample are slightly displaced since the nitrite absorbance registers slightly earlier on the chart recorder because of a shorter flow length for the sample.

