Plant stable isotope ratios (^{15}N) as an indicator of waste water nitrogen uptake

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ABSTRACT

An investigation was conducted into the processes that result in waste water N enrichment in ^{15}N and also the use of this phenomenon in tracing the source of plant N in the environment. A study of ^{15}N enrichment in various stages of a treatment plant was carried out at the Horotiu treatment plant with the Upper Waikato River and Northern Manukau Harbour systems being used in tracer studies in the environment.

Waste water NH_4 in the Horotiu treatment plant was found to become progressively enriched as it passes through the various treatment systems. The inorganic NH_4 in the raw dissolved raw effluent has been shown to be enriched by +4.1 $^{o}/_{oo}$. In the anaerobic pond organic nitrogen is mineralised and mixes with the inorganic NH_4 resulting in an increase in enrichment to +10.7 $^{o}/_{oo}$ due to the mass balance of the two materials. In the facultative pond nitrification and possibly NH_3 volatilisation further enriches the NH_4 to +13.3 $^{o}/_{oo}$.

In the Upper Waikato River *Lagarosiphon major* and *Enteromorpha nana* were used in a ^{15}N tracer study of the source of plant N due to their presence in both natural and waste water contaminated sections of the river. Ammonium is the major source of N available to the two plants and is derived from treated waste water and Lake Taupo NH_4. The ^{15}N abundance of waste water was +7.9 $^{o}/_{oo}$ enriched, while the Lake Taupo source was estimated using the values for a range of upstream plants to be near atmospheric natural abundance (ie. 0.0 $^{o}/_{oo}$). Isotopic fractionation during the assimilation of ammonium in the river appears to be low, this has enabled plant ^{15}N abundance to be used as indicator of source mixing. A two source mass balance mixing model has been used to determine the origin of N in the two species and indicates that 50 to 100 % of plant nitrogen is from waste water

ABSTRACT

Treated waste water ammonium has previously been found to be naturally enriched in ^{15}N, an investigation was conducted to identify the cause of this enrichment and it's potential use as a natural tracer of biological processing. A study was carried out in the Horotiu Treatment Plant to examine the ^{15}N enrichment of waste water NH_4 in various systems and isotopic fractionation during the processing of nitrogen. The Upper Waikato River and Northern Manukau Harbour were used in studies investigating the impact of enriched waste water NH_4 on plant N isotopic ratios in the environment.

Waste water NH_4 in the Horotiu Treatment Plant was found to become progressively more enriched as it passes through various treatment systems, this has been attributed to the enriched ^{15}N abundance of the substrate and various processes that interact with N at each level. Raw waste water from the nearby Affcco freezing works enters the treatment plant with a low NH_4 concentration (12.7 mgl^{-1}) derived primarily from blood and faeces and has a ^{15}N enrichment of $+4.1^{o}/_{oo}$. In the anaerobic pond protein is mineralised and mixes with the existing NH_4 to produce a much higher NH_4 concentration (106 mgl^{-1}) without significant isotope fractionation, so that the waste water NH_4 enrichment now resembles the value of the new substrate (ie.$+10.7^{o}/_{oo}$). In the facultative pond nitrification and possibly NH_3 volatilisation further enrich ^{15}N abundance of NH_4 to $+13.3^{o}/_{oo}$, this is the level of enrichment in waste water that is discharged from the treatment plant.

In the Upper Waikato River *Lagarosiphon major* and *Enteromorpha nana* were used in a ^{15}N tracer study to determine the source of plant nitrogen downstream of the Taupo Borough Pollution Control Plant, discharge into the river. Treated waste water and Lake Taupo, are the major sources of N in the river, with NH_4 being the predominant form available to plants. The ^{15}N abundance of waste water NH_4 was found to be $+7.9^{o}/_{oo}$ enriched, while the isotopic ratio of Lake Taupo source was estimated from the range of isotopic

values found in upstream plants to be near 0.0‰. Isotopic fractionation during assimilation appears to be small, this has enabled the use of a two source mixing model, using the ^{15}N abundance values from plants and the two N sources, to show that 50 to 100% of plant nitrogen in the downstream section of the river is from waste water (depending on the site).

In the Northern Manukau Harbour an attempt was made to apply ^{15}N methodology to tracing the impact of waste water NH_4 (the predominant source of NH_4 in the harbour) on plant nitrogen. Ammonium is the major form of N assimilated by plants in the northern harbour. Sediment and plant N (from Gracillaria) indicated that there is a significant fractionation during assimilation, which highest near the waste water outfalls from the Manukau treatment plant, where plant N is -1 to+ 9‰ enriched compared with NH_4 c.+20‰. The degree of fractionation indicates that only a small portion of the NH_4 in the harbour is assimilated. A study of SOM indicated that ^{15}N isotopic ratios in the treatment plant were different to the values in the southern harbour (ie. c. -5‰ compared with +12‰). A mass balance model was used to indicate the mixing of the two sources of SOM in the harbour. Results show that c. 30% of the SOM in the northern harbour is derived from the treatment plant, decreasing to 7% in the harbour mouth where a further mixing with SOM from the southern harbour is apparent.

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percentage of the total nitrogen in the substrate and product at time t and in the delta notation by $\delta^{15}N_s$ and $\delta^{15}N_p$. The amount of substrate and product at set intervals during the reaction is shown by N_s the substrate remaining at time t and N_p the accumulated product at the same time both of which are arbitrary values . Product and substrate is removed and added at a constant rate throughout the time period of the reaction so that the overall quantities of substrate and products remain constant. The isotope effect remains constant throughout, and therefore the fractionation in the substrate and product is also constant. Thus after time t = 3 there will be no changes as all factors that may cause changes in ^{15}N are constants.

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Alpha (α): Equal to the reciprocal of Beta ie 1 / β (see Beta). The values obtained generally range from 1.05 - 1.00.

Atom % ^{15}N : The absolute abundance of the ^{15}N isotope, by convention Atom % is described as the percentage of atoms which occurs for the heavier isotope (ie for Nitrogen this is ^{15}N). The general expression is shown by the following example for atmospheric N_2 : .3663 Atom % ^{15}N in Atmospheric N_2 .

Beta (β) : Beta is the term used to describe the kinetic isotope effect, and is determined by dividing the isotopic ratio of the substrate into the isotopic ratio of the products. The value obtained is usually in the range of 1.00 - 0.95.

Delta : This is the term most commonly used to describe the isotopic status of a sample. It is the difference between the sample and the natural abundance of ^{15}N given in parts per thousand ($=^{o}/_{oo}$).

Depleted : This is a term used to describe the reduced ^{15}N concentration (with regard to ^{14}N) of a product material compared to the ^{15}N concentration in the substrate material, for example a product material having .3650 Atoms % ^{15}N and the substrate material .3700 Atoms % ^{15}N. The product contains .0050 Atoms % ^{15}N less than the substrate it was produced from.

Effluent : Waste water that has passed through a system in a sewage treatment plant.

Enriched : This is a term used to describe the increased ^{15}N concentration (with regard to ^{14}N) of a product material compared to the ^{15}N concentration in the substrate material, for example a product material having .3700 Atoms % ^{15}N and the substrate material .3663 Atoms % ^{15}N. The product contains .0037 Atoms % ^{15}N more than the substrate it was produced from. The term is most commonly used when comparing the ^{15}N concentration of a material to atmospheric N_2.

Fractionation : The name given to the result of processes that favour a lighter (or heavier) isotopic molecule over a heavier (or lighter) one during biological and chemical processes. The products produced will be either lighter (or heavier) than the substrate it was produced from.

Heavier Isotope Molecule : The name given to a molecule that incorporates an atom containing the ^{15}N isotope.

Influent : Waste water that is entering a system in a sewage treatment plant.

Isotope Composition : The relative distribution of the isotopes of a given element, expressed in Atom % of the heavier isotope (ie. for N_2 in the atmosphere this is .3663 Atom %^{15}N at natural abundance).

Isotope Effect : This is the process that gives rise to fractionation. An isotopic effect results in the descrimination between the isotopes of the same element. Thus the expression of isotopes in the products will differ to that which is found in the substrate.

Isotope Ratio : This is the ratio of the heavier isotope to the lighter isotope (eg at natural abundance $^{15}N : {}^{14}N = 1 : 272$).

Kinetic Effect: An isotopic effect caused by differences in the reaction rates of isotopes of the same element but with different masses.

Lighter Isotope molecule : The name given to a molecule that incorporates an atom containing the ^{14}N isotope.

Magnitude : The size of a kinetic isotopic effect (= difference from β = 1.000)

M. S. : An abbreviation for mass spectrometer.

Mass Spectrometer : A machine that separates isotopes using electrical and magnetic fields by mass and charge.

Natural Abundance : Generally used to depict the ratio between stable isotopes of an element from a reference substance which is normally given the delta value of 0. More specifically in the case of nitrogen this describes the atmospheric ratio of the dinitrogen molecule between the two isotopes ^{15}N and ^{14}N which is 272 : 1 (this equals 0.3663%) which is used as the standard reference material. Alternatively other materials that have a similar isotopic ratio are said to be near (or equal) to natural abundance.

per mil ($^{o}/_{oo}$) : The units for delta values (see delta for explanation of delta values).

Products : The nitrogen containing material that is produced as a result of a nitrogen reaction (eg nitrification) or by a physical process (eg absorption)(see also substrate).

Receiving Body : The system (eg. a river) in which treated effluent is discharged.

Standard Reference : The standard reference for nitrogen is the dinitrogen molecule in atmospheric air. This has .3663 Atom % ^{15}N (= 0.0 $^{o}/_{oo}$ ^{15}N) and is the value that other nitrogen containing materials are compared with.\

Standard Error (SE) : The variation in the standard deviation with regard to the sample size. Calculated by multiplying the standard deviation by 1.96 and then dividing by the square root of the sample size.

Substrate : The nitrogen containing material that provides the nitrogen source for a physical process (eg absorption) or a reaction (eg nitrification) (see also product).

Suspended Organic Matter (SOM) : Organic matter comprising of phytoplankton, bacteria and sediments in the water column.

Working Reference : A material that has a natural abundance value that approximates that of air (.3663 Atoms % ^{15}N) or is similar to the range of values expected to be obtained from a run of samples, and is used in M. S. analyses for convenience (eg Urea). The working reference has been calibrated against atmospheric dinitrogen and has a known delta $^{o}/_{oo}$ ^{15}N value and variation.

Chapter 1

Nitrogen Stable Isotopes in the Environment

1.1 Preamble

There are various ways of assessing a nutrient (or pollutant) source impact on a receiving body. In the past this has usually been carried out by comparing upstream and downstream water properties, biota or aesthetic values. In these situations indirect and circumstantial evidence may be used to determine the effect of the nutrient source on the water body. The use of stable isotope information enables the possibility of introducing direct evidence concerning the effect of a particular nutrient downstream from a discharge, and hence the purpose of this study is to demonstrate how this can be achieved for nitrogen in treated effluents discharged into the Upper Waikato River and Northern Manukau Harbour respectively.

In the environment the stable isotopes of an element (such as nitrogen) occur at a particular ratio, that can be considered to be constant, with only small deviations (plus or minus) from the natural abundance. The natural abundance of stable isotopes are determined by predictable characteristics (eg. reaction rates), and are known, or can be predicted by a knowledge of isotope chemistry. This necessitates an understanding of isotope effects during reactions and thus the following chapter reviews general isotope chemistry and more specifically ^{15}N isotope chemistry, and it's application to^{15}N stable isotope studies, as well as revisiting biological processes where these techniques can be used

All elements exist in a variety of forms called isotopes, some of which are radioactive and some are stable. Nitrogen occurs as radioactive isotopes, (^{12}N, ^{13}N, ^{16}N and ^{17}N with half lifes of 0.0125s, 605s, 7.38s and 4.14s) and as stable isotopes, which are non radioactive and are identified by the differing

neutron number (ie. atomic weights). Nitrogen is an element for which there are two common stable isotopic forms ^{14}N and ^{15}N and their concentration and behaviour in the environment are relatively predictable but little studied. The common isotope ^{14}N occurs at 99.6337 atom % while the heavier isotope ^{15}N occurs at 0.3663 atom % in air. Small deviations in this value for ^{15}N may be measured in the environment and are given as delta units (δ) (parts per thousand $^{o}/_{oo}$), which are deviations (plus or minus) from the natural abundance. Most differences are in the order of -5 to +10 $^{o}/_{oo}$ and are measurable to at least 0.1$^{o}/_{oo}$ by modern mass spectrometers.

1.2 Historical Aspects of Stable Isotopes

The discovery of stable isotopes derived from the work on radioactive substances during the early part of this century. Early researchers (Boltwood, 1906; 1907; Marekwald, 1910) were working on the chemistry of the elements and found that certain radioactive substances had identical chemical properties but differed in their radioactive properties. This lead to the conclusion that this was probably of general occurrence, and that the isotopes of the elements differed in their atomic weights as well. Shortly after these findings, Thomson (1911) used a mass spectrograph to measure positive ions, which lead to the discovery of the two isotopes of neon (^{20}Ne and ^{22}Ne). The building of new instruments (mass spectrometers) assisted the progress that was being made (Dempster, 1918) so that by 1924 more than 50 stable isotopes had been discovered. The ^{15}N isotope was discovered in 1929 (Naude, 1929) and the first mass spectrometer work on measuring ^{15}N was carried out by Vaughan and co-workers (Vaughan et al,1934).

After the initial burst of discoveries, attention was focused towards the study of the light isotopes (ie H, D,^{12}C,^{13}C,^{14}N,^{15}N,^{16}O and ^{18}O) by Nier, Urey, Rittenberg and others leading to the quantitative principles of isotopic

fractionation (eg. Urey, 1947). About this time the first commercial mass spectrometers became available and this was followed by early work on oxygen and carbon isotopes by geochemists. Rittenberg et al (1939) determined methods suitable for mass spectrometer analysis of biological samples. Using these methods Schoenheimer and Rittenberg (1939) discovered variations in the ^{15}N / ^{14}N ratios of amino acids .

The ^{14}N / ^{15}N ratio in atmospheric dinitrogen was found to be 272 ± 0.3 (Junk and Svec, 1958) and this has been generally accepted as the standard value for N_2 in air (Fuller, 1959). Various studies have shown that there is little variation in the isotope ratio for different locations (Chackett et al, 1950; Chackett, 1951; Hoering, 1956 and Mariotti, 1983) or altitude (Dole et al, 1954). The most recent study showed a maximum variation of ± 0.0004 % (Mariotti, 1983). The precision to which the measurement of atmospheric dinitrogen has been made has since improved and the isotope ratio has finally been accepted as being 1:272 (ie 0.3663 ± .0004 Atom % ^{15}N) (Mariotti, 1983).

Work by Urey (1947) and Bigeleisen (1949b) suggested that source-product isotopic variation might be useful in following chemical reactions. The first evidence that $^{15}N/^{14}N$ varied in natural samples was provided by White and Yagoda (1950). Their work was carried out on nitrogen occluded in uranium minerals and indicated a relationship between the geological age and the ^{15}N content, older materials having a higher ^{15}N content than younger materials. Hoering's work on biological material showed that there were measurable differences in material from different origins (Hoering, 1955). This study was quickly followed by Parwel and coworker's studies on ^{15}N /^{14}N abundance (Parwel et al, 1957) and further supported by work on the isotope ratio in rainwater ammonia and nitrate (Hoering,1957) . This latter study is significant in that it is the first to attempt to trace the source of ^{15}N variation in a particular form of nitrogen.

1.3 Isotopic Effects

1.3.1 Introduction

Stable isotopes differ in mass as a result of different neutron number. The electron shells are identical which results in similar chemical properties, but the mass difference and differing nuclear spin give them differing physical properties which results in small changes in their behaviour, thus the isotope composition of the products may differ slightly to that of the substrate. This phenomena is called the isotope effect and is a characteristic of most reactions.

Discrimination results from the physicochemical inequalities of the isotopes and may affect either the progress rate (kinetic effects) or the energy state (thermodynamic effects) of a molecule. As a consequence, a fractionation effect is produced that results in a different distribution of the isotopes. For example, discrimination during a reaction increases the concentration of one isotope relative to another in one substance while decreasing it in the other. Hence an understanding of isotopic effects is an important factor in our interpretation of stable isotope tracer studies.

1.3.2 Kinetic

The Kinetic Isotope Effect is the result of differences in the reaction rates of different isotopes of the same element. Molecules carrying the smaller mass have a greater velocity and are more mobile than compounds carrying the heavier isotope. Hence during physical processes (eg diffusion) the lighter molecule will move more rapidly than the heavier molecule for the same expenditure of energy. This is described by the kinetic energy equation, which states that K.E. = 1 / 2 mv^2. Kinetic energy is equal to half of the molecules mass, multiplied by it's velocity squared. The K.E. is proportional to the

absolute temperature (K), hence as K.E. will be constant for all the isotopic compounds in a substance, then the velocity of the larger mass is slower.

A second factor, chemical fractionation, occurs because the chemical bonds formed by the heavier isotopic molecules are stronger than those formed by the lighter isotopes. This is due to the vibrational frequency in the heavier isotope being lower than in the lighter isotope, hence the probability of breaking a bond is greater. The effect depends on the differences in the kinetics of the isotopic forms, not on the kinetics of the reaction (ie it depends on the difference in activation energy of the different isotopes, not on the absolute value of activation energy for the reaction). This results in the products of a reaction being depleted in the heavier isotope and enriched in the lighter isotope. In contrast the inverse occurs in the substrate with the lighter isotope being depleted more quickly than the heavier isotope. The kinetic isotope effect decreases as temperature increases, when the temperature is very high (ie T > 800 - 1000 K) then the activation energy becomes insignificant, and the isotope effect is determined only by the ratio of the masses.

1.3.3 Thermodynamic

The thermodynamic effect is the results of small differences in the amount of free energy in isotopic molecules or compounds. A molecule (or compound) that contains a heavier isotope consequently has a smaller reserve of free energy than an equivalent compound with an isotopic lighter form. From observations of the atomic and molecular spectra of protium and deuterium in their various compounds it has been concluded that from the differences in their free energies an isotope effect will be no more than 0.5% of the total effect (Bigeleisen, 1965). Thus we can ignore the Thermodynamic effect .

1.3.4 Delta (δ)

The isotopic abundance of a material may be expressed in atom %, or in delta notation which is the measure of enrichment or depletion with respect to a standard substance (for nitrogen this is atmospheric N_2). Values are expressed in parts per thousand, ($^{o}/_{oo}$) plus or minus. For nitrogen (Eq. 1.1) the materials atom % ^{15}N value is divided by the natural abundance of atmospheric dintrogen and multiplied by a thousand over one, with the subtraction of a thousand to determine the difference from the standard. This may be approximated by using only the ^{15}N values (Eq. 1.2) instead of using ^{14}N in the equation as well.

$$\delta = \frac{^{15}N/^{14}N\ (\text{sample})}{^{15}N/^{14}N\ (\text{natural abundance})} \times \frac{1000}{1} - 1000 \qquad \text{(Eq. 1.1)}$$

$$= \frac{^{15}N\ (\text{sample})}{.3663} \times \frac{1000}{1} - 1000 \qquad \text{(Eq. 1.2)}$$

For example a material that has .3700 Atom % ^{15}N. The $\delta^{15}N$ in parts per thousand is calculated as follows:

$$\delta = \frac{.3700 / 99.6300}{.3663 / 99.6337} \times \frac{1000}{1} - 1000 = 10.1014\ ^{o}/_{oo}$$

or approximated by

$$\delta = \frac{.37}{.3663} \times \frac{1000}{1} - 1000 = 10.1010\ ^{o}/_{oo}$$

The delta values for a range of nitrogen containing materials are shown in Appendix A1 - A6.

1.3.5 Beta (β)

The magnitude of the isotope effect expressed during a reaction is important in determining the isotope ratio in the products. In this study the Greek letter β has been used when talking about the magnitude, however in literature both β and α are used (and will be explained later). β is taken to be equal to the isotope ratio of the products divided by the values for the substrate (Eq. 1.3)

$$\beta_{p/s} = R_p / R_s \qquad \text{(Eq. 1.3)}$$

where R_p is the isotope ratio of the product which occurs at the instantaneous start of a reaction (t = instantaneous) and R_s is the isotope ratio of the substrate at the same time. This can be expressed for nitrogen as:

$$\beta_{p/s} = \frac{^{15}N / ^{14}N \text{ (products)}}{^{15}N / ^{14}N \text{ (substrate)}} \qquad \text{(Eq. 1.4)}$$

In practise the β value can be approximated by using the existing isotopic abundances of the substrate and accumulated product at the time of collection.

In literature both β and it's reciprocal α have been used to describe the Kinetic Isotope Effect. However there appears to be much confusion over their usage.

(a) $\alpha = \frac{^{14}N/^{15}N \text{ (Products)}}{^{14}N/^{15}N \text{ (Substrate)}}$ Mariotti et al (1981)

(b) $\alpha = \frac{^{15}N/^{14}N \text{ (Substrate)}}{^{15}N/^{14}N \text{ (Products)}}$ Horrigan et al (1990)
Wada and Hattori (1978)

(c) $\beta = \frac{^{15}N/^{14}N \text{ (Products)}}{^{15}N/^{14}N \text{ (Substrate)}}$ Delwiche and Steyn (1970)
Shearer and Kohl (various) and in this study

(d) $\beta = \frac{^{14}N/^{15}N \text{ (Substrate)}}{^{14}N/^{15}N \text{ (Products)}}$ As yet not applied

The different α and β expressions used are rearrangements of the same formula and therefore obtain the same answer, or it's reciprocal:

eg. If the substrate nitrogen isotope % content is: R_s ^{15}N: .3700 ^{14}N: 99.63
and the products nitrogen isotope % content is: R_p ^{15}N: .3600 ^{14}N: 99.64
then:

(a) $\alpha = \frac{^{14}N/^{15}N \text{ (Products)}}{^{14}N/^{15}N \text{ (Substrate)}} = \frac{99.64 / .36}{99.63 / .37} = 1.02788$

(b) $\alpha = \frac{^{15}N/^{14}N \text{ (Substrate)}}{^{15}N/^{14}N \text{ (Products)}} = \frac{.37 / 99.63}{.36 / 99.64} = 1.02788$

(c) $\beta = \frac{^{15}N/^{14}N \text{ (Products)}}{^{15}N/^{14}N \text{ (Substrate)}} = \frac{.36 / 99.64}{.37 / 99.63} = 0.97288$

(d) $\beta = \frac{^{14}N/^{15}N \text{ (Substrate)}}{^{14}N/^{15}N \text{ (Products)}} = \frac{99.63 / .37}{99.64 / .36} = 0.97288$

with

$$\beta = 1 / \alpha$$

$$0.97288 = 1 / 1.02788$$

$$= 0.97288$$

β generally has a value between 1.000 and 0.970. 1.000 indicates that there is no fractionation (ie. the isotopic ratios of the products and the substrate are equal) and values < 1.000 show the degree of the isotopic effect that is expressed (the lower the value the greater the effect). Maximum fractionation is expressed when the amount of product is small relative to the substrate. Minimum fractionation is occurs when all the substrate has been converted into the product

In a closed system (eg. Table 1.1) where all the substrate is eventually consumed the isotopic abundance of the products and the substrate increases as the reaction proceeds towards completion. The product made has a lower delta value than the substrate which it comes from, however the isotope abundance of the substrate and product both change over time, reflecting the isotopic effect. As more and more product is removed, the isotopic effect results in an increasing delta value in the substrate, however as the ^{15}N concentration increases in the substrate increases fractionation will decrease and the β value will approach 1.000.

In open systems where the substrate is maintained at a high level with respect to the products, and both are added or removed at constant rates (ie. Table 1.2) a large isotopic effect will be observed, β will remain constant, unless of course there are changes in the isotopic composition of the substrate.

t	N_s	Atom% $^{15}N_s$	$\delta^{15}N_s$	N_p	Atom% $^{15}N_p$	$\delta^{15}N_p$	β	$\delta_{s,t} - \delta_{s,o}$	$\delta_{p,t} - \delta_{s,o}$
0	50.0	.36655	+1.5	0.0	-	-	-	0.0	-
1	40.0	.36813	+5.0	10.0	.36172	-12.5	.9826	+3.5	-14.0
2	30.0	.36886	+7.0	20.0	.36383	-6.7	.9864	+5.5	-8.2
3	20.0	.36941	+8.5	30.0	.36514	-3.2	.9884	+7.0	-4.7
4	10.0	.36996	+10.0	40.0	.36607	-0.6	.9895	+8.5	-2.1
5	0.0	-	-	50.0	.36655	+1.5	-	-	0.0

Table 1.1: Theoretical example of the fractionation that results from the isotope effect in an closed system where all of the substrate $_s$ is converted to product $_p$ in an arbitrary time t = 5 with the isotope effect β. The fractionation that occurs in the substrate and products is shown by Atom % $^{15}N_s$ and Atom % $^{15}N_p$ the ^{15}N percentage of the total nitrogen in the substrate and product at time t and in the delta notation by $\delta^{15}N_s$ and $\delta^{15}N_p$. The amount of substrate and product at set intervals during the reaction is shown by N_s the substrate remaining at time t and N_p the accumulated product at the same time both of which are arbitrary values. The product is consistently depleted in ^{15}N compared with the substrate throughout the reaction until all the substrate is converted to product. The initial product is depleted compared with the substrate with both becoming increasingly enriched in ^{15}N compared with their initial values until the substrate is all used up and the product is equivalent to the initial substrate δ value. Changes in the substrate $\delta_{s,t}$ and product $\delta_{p,t}$ from the substrate enrichment at time t = 0 ($\delta_{s,o}$) are also shown to indicate that although the values deviate from the initial substrate value, the value is eventually restored when all the product is produced.

t	N_S	Atom%$^{15}N_S$	$\delta^{15}N_S$	N_p	Atom%$^{15}N_p$	$\delta^{15}N_p$	β
0	$1x10^8$	.36655	+1.5	9.5	.35740	-24.3	.975
1	$1x10^8$	.36655	+1.5	9.5	.35740	-24.3	.975
2	$1x10^8$	.36655	+1.5	9.5	.35740	-24.3	.975
3	$1x10^8$	.36655	+1.5	9.5	.35740	-24.3	.975

Table 1.2 : Theoretical example of the fractionation that results from the isotope effect in an open system where some of the substrate $_s$ is converted to product $_p$ in an arbitrary time t = 3 with the isotope effect β. The fractionation that occurs in the substrate and products is shown by Atom % $^{15}N_s$ and Atom % $^{15}N_p$ the ^{15}N percentage of the total nitrogen in the substrate and product at time t and in the delta notation by $\delta^{15}N_s$ and $\delta^{15}N_p$. The amount of substrate and product at set intervals during the reaction is shown by N_s the substrate remaining at time t and N_p the accumulated product at the same time both of which are arbitrary values . Product and substrate is removed and added at a constant rate throughout the time period of the reaction so that the overall quantities of substrate and products remain constant. The isotope effect remains constant throughout, and therefore the fractionation in the substrate and product is also constant. Thus after time t = 3 there will be no changes as all factors that may cause changes in^{15}N are constants.

1.4 ^{15}N Natural Abundance Tracer Studies

1.4.1 Introduction

The use of nitrogen stable isotopes in natural abundance tracer studies is based on the relatively constant ratio of ^{15}N in the natural environment (with only small deviations from the natural abundance) and their slightly different behaviours. Thus any biological compound that has an isotopic ratio different to natural abundance has the potential to be used as a tracer. Materials that are naturally enriched (or depleted) can be used as tracers of the fate of nitrogen and / or nitrogen processing, as it moves through a system. This of course assumes that the N-isotopic composition of the material is sufficiently different from the system under investigation, and that it maintains it's isotopic identity throughout all transformations, or the size and direction of these transformations are known. The change that occurs in the isotope ratio to distinguish it from the background values enables the calculation of the extent to which the labeled-nitrogen has impacted the system.

1.4.2 Process Studies

1.4.2.1 Introduction

Several methods have been developed that use stable nitrogen isotopes as tracers of the reactions that are involved in the nitrogen cycle. There are two main methods : substrate enrichment (or depletion) (Delwiche and Steyn, 1970; Bergersen and Turner, 1983; Ledgard et al, 1985 and Gleens et al, 1991) and natural abundance studies (Kohl et al, 1971; Blackmer and Bremner, 1977; Peters et al, 1978; Ledgard et al, 1985; Hermes et al, 1985; Bottcher et al, 1990 and Hogberg, 1990a).

The first method using an enriched (or depleted) substrate has been applied particularly in the study of N_2-fixation by plants (Bergersen and Turner ,1983; Ledgard et al ,1985; Myrold and Tiedje ,1986; Witty ,1983 and Shearer and Kohl ,1987). This method is not of particular relevance to this study so will not be mentioned further.

The study of variations in natural abundance can be useful in investigations in biogeochemical, physical, physiological, chemical and biochemical reactions. ^{15}N /^{14}N ratios are generally used in determining the source and possible sinks and other reactions that may have occurred during the transformation of the nitrogen. This method relies on the mass-balance of all isotopes. As all the substance must be either substrate or product (ignoring intermediates) the combined isotope composition of the substrate and the product will equal the isotope composition of the substrate before any product is made.

Reactions that brings about changes to a nitrogen compound or form, will have a β value associated with each set of environmental conditions. The main processes that affect NO_3 and NH_3 are reviewed in this section, along with the experimentally determined, apparent β's for the reactions involved.

1.4.2.2 Physical Processes

NH_3 / NH_4 Equilibrium

Urey (1947) showed that the isotope effect attributable to the NH_4 and NH_3 equilibrium is small and varied between : β = 0.9960 to 0.9921.

Diffusion

Diffusion of ions and molecules in solution is thought to have little effect on isotope fractionation (Shearer and Kohl, 1986). The ion's (or molecule's) establishment of it's observed configuration is the result of it's electronic

surface potential which is practically independent of the isotope composition (Shearer and Kohl, 1986). Thus virtually no isotope fractionation will result from the diffusion of ions or molecules through a liquid medium.

Diffusion of gases through gases can be expected to result in a significant isotope fractionation (Shearer and Kohl, 1986). This isotope effect is equal to the square root of the reduced masses (Farquhar et al, 1983).

Ion Exchange

Discrimination during ion exchange has been shown to produce only a small fractionation of N isotopes. Cation exchange discrimination varied from β = .9995 to .9986 (Delwiche and Steyn, 1970, Karamanos and Rennie, 1978). While anion exchange ranged from β = 1.0028 to 1.0016 (Delwiche and Steyn, 1970). Therefore the fractionation attributable to ion exchange can be considered to be negligible.

NH_3 Volatilisation

Ammonium may be lost from a liquid as the result of a combination of chemical and physical reactions known collectively as NH_3 volatilisation. The pathway requires the diffusion of NH_4 through water and the formation of NH_3 (from NH_4) which is lost to the atmosphere. The significance of volatilisation in removing N from solution depends on the equilibrium that is established between NH_3 and NH_4, which in turn is determined by several factors: pH, wind speed and temperature. Hence NH_3 volatilisation may result in only a minor loss of N, or it can represent the dominant fate of NH_3 in a system, therefore isotope discrimination associated with this process may be important in assessing the cause of isotopic enrichment in a sewage treatment plant (Freney et al, 1983).

The first factor (pH) determines the proportion of NH_3 to NH_4 in the solution. When the pH is <6 the equilbrium reaction favours NH_4 and no NH_3 is

present, at > 6 ammonia is produced and at pH 8 is a significant in solution (ie c. 10%). and at pH >10 all the NH_4 is converted to NH_3.

The second factor (wind speed) increases the flux of ammonia from the aqueous to the gaseous phases. Air adjacent to the waste water is removed, along with volatilised ammonia, thus the concentration gradient between the two phases is increased.

The last factor (temperature) affects the dissociation of compounds into ions, and also it's solubility. When the solution temperature increases dissociation increases and solubility decreases, the converse is true when the temperature decreases.

The rate at which NH_3 is volatilised from a solution is particularly important in determining the isotope fractionation that the pathway produces. At high rates of NH_3 loss from a solution the isotope discrimination would be expected to be maximised for all the processes, while when the loss is low the actual fractionation of the constant (the conversion from aqueous to gaseous NH_3) will be approximated, as all the other processes will have minimal effect.

The size of the isotope effect associated with volatilisation has been established for various conditions (Mariotti, 1982). Under equilibrium conditions the isotope effect varied between β = 0.9761 to 0.9739 depending upon temperature. However under non-equilibrium conditions the isotope effect varied between β = 0.9917 to 0.9694 depending on NH_4 concentration and NH_3 removal. When the removal rate was high the isotope effect was larger. Low concentration of NH_4 resulted in only a small isotope effect. Thus the volatilisation isotopic effect observed is the result of the cumulative effect of several processes that interact in the changing of $NH_{4\,(aq)}$ to $NH_{3\,(g)}$.

1.4.2.3 Biological Processes

N_2 fixation

The biological process that converts the dinitrogen molecule N_2 to ammonia is called nitrogen fixation. This process is only carried out by bacteria that exist as free living microbes or live in a symbiotic association.

Fractionation that is attributable to the isotope effect during N_2 fixation ususally lies in the range β = 1.0020 - 0.9980 (Shearer and Kohl, 1986). Studies have been carried out on a wide variety of organisms and parts of organisms. The large change in bonding from N N to NH_3 as N is reduced would be expected to give a large isotope effect if the N_2 reduction steps were the most important in determining the rate of the reaction. However it would seem that the enzyme association is more important in determining the rate of the reaction (Hoering and Ford, 1960). Experimental determination of the apparent β associated with N_2 fixation is shown in Table 1.3. The range in β is due to the measurement of more than one species of organism by the authors.

N_2 - fixation fractionation

β	References
0.9969 - 0.9956	Delwiche & Steyn (1970)
1.0010 - 0.9990	Hoering and Ford (1960)
1.0180 - 1.0010	Kohl and Shearer (1980)
1.0017 - 0.9980	Kohl and Shearer (1986)
0.9992 - 0.9976	Mariotti et al (1980)
1.0037 - 0.9960	Shearer & Kohl (1986)
1.0000 - 0.9966	Steele et al (1983)

Table 1.3: Experimental β values for the fractionation that is associated with N_2 fixation. A range is given for the value to indicate the variation under different conditions and species.

Denitrification

Dentirification is the biological process that reduces nitrate to gaseous nitrogen end products, usually N_2 and nitrous oxides. Nitrate serves as a terminal acceptor on an electron transport pathway, which initiates new acceptors further down eventually producing N_2 gas. At each stage the pathway is catalysed by different enzymes.

Results of several studies for determination of the fractionation associated with denitrification is shown in table 1.4. The level of observed isotopic effect (β) associated with denitrification in cultures and soils varies between β = 0.9980 and 0.9560 (Table 1.4). The size of β increases with higher electron concentration and decreases when the electron concentration is lower (Chien et al, 1977 and Mariotti et al, 1981) β also decreases as temperature increased (Mariotti et al, 1981).

Denitrification (NO_3 - N_2)	
β	References
0.9900 - 0.9670	Mariotti et al (1981)
0.9856 - 0.9793	Miyake and Wada (1971)
0.9760 - 0.9560	Richards and Benson (1961)
0.9866 - 0.9792	Wada et al (1975)
0.9980 - 0.9970	Wellman et al (1968)

Table 1.4: Experimental β values for the fractionation that is associated with denitrification. Changes in conditions produce the range of β values shown.

Nitrification

Nitrification is carried out by free-living bacteria that oxidise reduced nitrogen compounds. The first oxidation is ammonia to nitrite carried out by Nitrosomonas. Nitrite is subsequently oxidised to nitrate by nitrobacter.

Descrimination associated with the oxidation of NH_4 to NO_2 has been examined using pure cultures of nitrifying organisms (Delwiche and Steyn, 1970; Miyake and Wada, 1971; Shearer and Kohl, 2986 and Horrigan et al, 1990a). The observed range for β was from 1.0200 to 0.9620 (Table 1.5).

Nitrification		
Reaction	β	References
NH_4 - NO_2	1.0200 - 1.0170	Delwiche & Steyn (1970)
	0.9880 - 0.9840	Horrigan et al (1990a)
	0.9850 - 0.9750	Miyake and Wada (1971)
	1.0000 - 0.9620	Shearer & Kohl (1986)
NH_4 - NO_3	0.9820 - 0.9640	Delwiche & Steyn (1970)
	0.9875 - 0.9843	Horrigan et al (1990a)

Table 1.5 : Experimental β values for the fractionation that is associated with nitrification. A range is given for the value to indicate the variation under different conditions and species.

In soils the isotope effect for the oxidation of NH_4 to NO_2 has been observed to vary between β = 0.9881 -0.9718. In most soils the oxidation of NH_4 to NO_2 is much slower than the change from NO_2 to NO_3, therefore NO_2 does not accumulate. Thus the second oxidation would not be expected to contribute to the isotope effect due to the reaction going to completion. The concentration of NH_4 and NH_3 seems to have an important effect on the size of the isotope effect (Suzeki et al, 1974 and Mariotti et al, 1981). Both the limitations imposed by diffusion and the NH_4 / NH_3 equilibrium have a significant input into the

size of the fractionation that occurs. Mariotti et al (1981) showed that the β observed increased from 0.9728 to 0.9881 during the course of an experiment as the concentration of NH_4 decreased (agrees with the result predicted in a closed system). Table 1.5 shows the experimentally determined apparent β for nitrification from a variety of studies.

Assimilation

Assimilation of nitrogen is the general term for biological conversion of fixed nitrogen compounds to organic nitrogen. A growing plant assimilates nitrate or ammonia converting it into protein, nucleic acids and other nitrogenous compounds. An animal assimilates amino acids, building them into proteins and other biological polymers. The pathway is a complex arrangement which for the purpose of this study will not be discussed further.

The observed isotopic fractionation (β) associated with the assimilation of NO_3 and NH_4 has been shown to vary in experiments between β = 1.0098 and 0.9740 (Table 1.6) for various environmental conditions. In plants the magnitude of the isotope effect depends on illumination and NH_4 concentration (Wada and Hattori, 1978), NO_3 concentration and plant age (Mariotti et al, 1982). Thus it can be concluded that there will be little fractionation for actively growing plants in high light intensity and moderate NO_3 or (NH_4) concentration.

Reaction	Assimilation β	References
Absorption: Plant		
NO_3	1.0000 - 0.9822	Mariotti et al (1982)
	1.0000 - 0.9740	Shearer & Kohl (1986)
	1.0000 - 0.9997	Shearer & Kohl (1986)
	1.0010 - 0.9978	Mariotti et al (1980)
NH_4	1.0000 - 0.9740	Shearer & Kohl (1986)
Assimilation: Higher Plant		
NO_3	1.0007 - 0.9978	Shearer & Kohl (1986)
Assimilation: algae		
NO_3	1.0000 - 0.9815	Wada et al (1975)
	0.9993 - 0.9775	Wada & Hattori (1976)
	0.9991 - 0.9982	Wada and Hattori (1978)
NH_4	1.0000 - 0.9904	Wada et al (1975)
	1.0098 - 1.0053	Wada & Hattori (1976)
	1.0073 - 0.9904	Wada and Hattori (1978)
NO_2	0.9986 - 0.9960	Wada and Hattori (1978)

Table 1.6: Experimental β values for the fractionation that is associated with absorption and assimilation of inorganic nitrogen by plants. A range is given for the value to indicate the variation under different conditions and species.

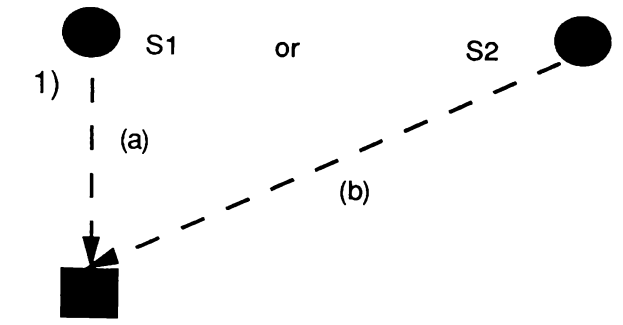
1.4.3 Source Studies

Stable isotopes have recently (since the 1970's) been used in the tracing of nitrogen in the environment at natural abundance levels. These studies rely on the use of small natural variations in ^{15}N and known isotopic effects and have been made over the five major ecological environments: Atmosphere; Terrestrial; Freshwater; Estuarine and marine. These studies have been useful in helping our understanding of the nitrogen cycle and it's reactions. However they have also revealed the complexity of the nitrogen cycle and the difficulty that can be encountered in interpreting the results of a study.

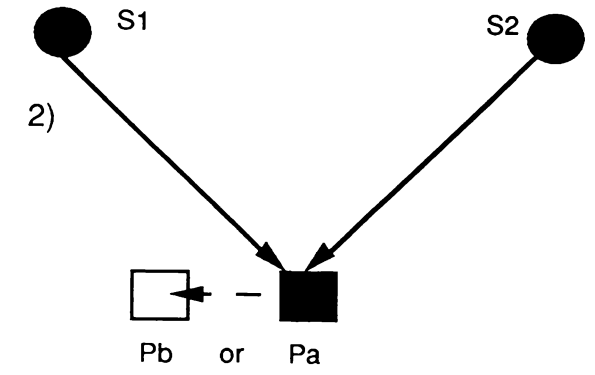
The use and interpretation of ^{15}N as a source indicator is depicted in figure 1.1, three examples are given, from the simplest case that of a single source to multiple sources. Case 1 is the type when a sample could have been derived from one of two different sources of known and different $\delta^{15}N$ values (eg N_2 fixation). If there is no fractionation then the products (P) must have the substrate S1 as it's source, however if isotopic fractionation occured then it's source is S2. An example of case one studies are food chains.

Published data by Macko et al (1982) on marine invertebrates, Minagawa and Wada (1984) marine invertebrates and invertebrates and Deniro and Epstein (1981) terrestrial invertebrates and mammals, indicate that organisms have a delta values that are related to there diet.

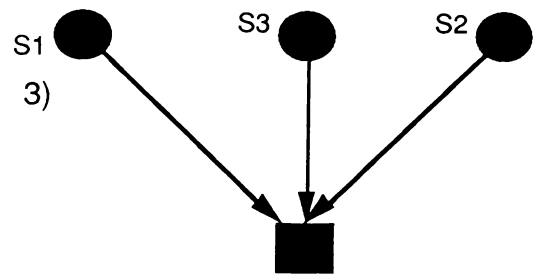
Several studies (eg. Miyake and Wada ,1967; Wada and Hattori, 1976 and Pang and Nriagu; 1977) have shown that there is an increase in isotopic enrichment for successive tropic levels. However this relationship is not very well defined, the study by Deniro and Epstein (1981) showed that there may be as much variation between organisms of the same species feeding on the same diet as there is between tropic levels.



Single source:
(a) without and
(b) with fractionation.
Either S1 or S2 is the substrate used to produce the products.



Dual sources:
(Pa) without and
(Pb) with fractionation.
S1 = 50% and S2 = 50% of the substrate to produce the products.



Multiple sources:
With fractionation:
When the contribution of each substrate to produce the products is given by; S1<S3<S2 or S1<S2<S3 .
Without fractionation:
When the contribution of each substrate to produce the products is given by; 100%=S1+S2+S3 where S1=S2, or100%=S3.

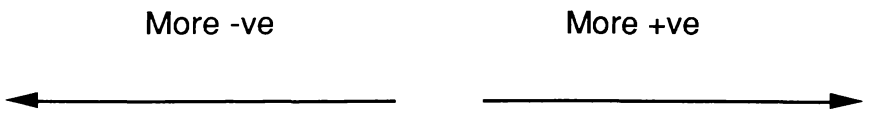


Figure 1.1. Diagrammatic representation of the use of natural variations in ^{15}N as an indicator of source of origin. S1-3 are sources with different isotopic ratios. The three examples show the effects on the products when one, two and three substrates contribute with and without fractionation to the products. Several possible ways of interpreting the products are shown (adapted from Owens, 1985).

In Case 2 (dual sources), two substrates contribute to the products (P). The delta of the products will lie in between the two substrate deltas with it's position depending on the contribution of the separate substrates and fractionation.

The potential effect of isotope fractionation may not necessarily hinder an interpretation completely. If enrichment or depletion occurs, the mass-balance of the isotopes must be maintained, therefore changes in the contributing source may be detectable. Case 2 studies are the most common type that is reported in literature. They differ from case 3 studies in that less information is required on the proportional contributions of the substrates. Case 2 has been extensively applied to coastal and estuary dissolved nitrogen and sediment studies (eg Sweeney et al, 1980; Sweeney and Kaplan, 1980b; Olson, 1981 and Cifuentes et al, 1988). Generally the studies have been used a consistent difference between two sources to enable the degree that each contributes to the products, to be tabulated.

Sweeney et al (1980) used a mass-balance mixing model equation to indicate sewage contribution in Californian coastal sediments. Their model linked the isotope composition and the nitrogen content in the sediments to the N sources. By comparing the natural abundance values of marine phytoplankton and sewage effluent it was shown that the proportion of sewage derived nitrogen varied between 25 and 75% in the Santa Barbara Basin, California (Sweeney et al, 1978 and Sweeney and Kaplan, 1980a). However the model used is sensitive to source changes that introduce large errors into the calculations.

In the study conducted by Mariotti et al (1984) they identified the origin of suspended organic matter components in the Scheldt estuary. The different natural isotope enrichments of the two endpoints (continental and marine) was used in the calculations. Their data for a winter survey showed a gradient between the two endpoint $\delta^{15}N$ values with intermediate sites having a $\delta^{15}N$ value in between the two extremes enabling determination of the contribution

of marine and freshwater particulate materials. However in the other surveys the $\delta^{15}N$ of the organic matter in the estuary was higher than either of the endpoints, suggesting that another source not present during the winter may make a significant contribution to the SOM during the rest of the year.

Case 3 (multiple sources) shows the situation when there are more than two sources. The proportion that each substrate contributes will determine the final product delta. However, if there is a fractionation effect in either multiple or dual source studies, their interpretations become rather ambiguous with the lighter substrate appearing to have contributed a larger proportion to the products than they actually have.

When three or more sources (case 3) contribute to an observed sample $\delta^{15}N$, the interpretation of their delta values are more complex due to the increasing number of sources and the combinations that are possible. These studies require additional information about the sources such as the isotopic effect associated with each source utilisation and the amount of each, that is available for use.

Multiple isotope tracing is one approach that has been tried to overcome the multiple source problem. Peters et al (1978) measured the nitrogen and carbon stable isotope ratios of sediment organic matter. They showed that there was a high correlation (mean $R^2 = .85$) between $\delta^{15}N$ and $\delta^{13}C$ values suggesting that this was the result of mixing of various marine and terrestrial materials with virtually no change in the overall fractionation by fixation, assimilation, denitrification and sedimentation.

Chapter 2

Methods for collecting and processing material for ^{15}N analysis

2.1 Preamble

The methods used for collecting and processing N - containing material for ^{15}N analysis needs to ensure that a representative sample is gathered, minimal N is lost during extraction and concentration of N, and that the isotope ratio measured is the true value for the substance. Nitrogen can be lost at every stage during processing (ie. collection, storage, oven drying, grinding, chemical analysis and N - concentrating procedures) prior mass spectrometer analysis.

A literature review of methods used in isotopic studies has been carried out. Errors that may occur due to incorrect sample preparation and / or handling have been mentioned and a set of methods using equipment that was available for this study has been determined to minimise their effects.

2.2 Methods Review

When collecting N - containing material it is important that the material is a representative sample. The N - content of a sample may vary, depending on what it represents, for example top soil is likely to be higher in N than B level soil, and leaf material will contain more N than the stem of the same plant. Thus it is important that the N - material collected is truly representative sample of the substance that is being assessed.

The time period and environmental conditions between collection and drying samples, can alter the dry matter content through respiration, which may result in a change in N - content. However few studies have been carried out to investigate the significance of storage time and conditions on N-content. Cochrane and Brown (1974) found that drymatter and crude protein content did not change when plant material was processed after 24 hours instead of half an hour. The study of Melvin and Simpson (1963) that a relatively large decrease in drymatter (8.4%) due to changes in environmental conditions resulted in a comparatively small drop in total N (0.1%).

Various drying methods (ie oven, freeze and microwave) have been used to halt chemical changes in materials and enable the assessment of drymatter content. The oven drying method is the most commonly used (as in this study), however care must be taken to ensure that the right drying temperature range is maintained. If the temperature of the oven is below 50-60 ^{o}C (ie. low) respiration may result in N loss (Mayland, 1968 and Smith, 1969), however if the temperature is too high (ie >70 ^{o}C) for prolonged periods the excess heat may cause the material to be degraded (Mayland, 1968 and Sharkey, 1970). Hence the oven temperature should be maintained between 60 and 70^{o}C to minimize N loss through oven drying.

After oven drying plant material is ground to enable a representative subsample to be made for chemical analysis. Depending on the volume of material this may be carried by one of various mills, or by simply using a Mortar and Pestal. In both grinding methods the ground plant material may electrostatically stick to the internal parts of the mill or pestal or be may escape into the atmosphere. The powdered material is generally less fibrous, and is therefore likely to have a higher nitrogen content, thus resulting in a decrease in the percentage N composition of the substance (Wetselaar and Farquhar, 1980).

The Kjeldahl and Combustion procedures are the major methods by which nitrogen is determined chemically, however both these methods may be prone

to loss of nitrogen under certain circumstances. In the case of the Kjeldahl method, the particular variation of this method used, often does not ensure that NO_3-N is converted to NH_4-N, and results in an undetermined amount of nitrate-N being reduced to ammonium-N (Bremner, 1965). The combustion method is generally considered to be superior to the Kjeldahl, with higher N values being obtained, however these values may be due to incomplete combustion and the formation of methane. The method has been found to over estimate the N content of certain substances (eg. natural oils, pyrimidines and amides of long fatty chains) (Van Meter et al, 1951) and underestimate other compounds especially heterocyclic compounds (which are difficult to burn) (Alford, 1952). In general for plant, animal and soil N none of these problems are encountered.

Loss of NH_4-N during distillation and concentrating prior to mass spectrometer analysis may be quite significant, errors during concentrating NH_4-N depend on the effectiveness of the trapping acid and the temperature during the evaporation procedure. The effectiveness of three trapping acids (ie. HCl, H_2SO_4 and H_3BO_3) have been compared for their ability to rapidly and efficiently concentrate large numbers of samples with minimum error in ^{15}N measurements (Reeder ,1984). Significant fractionation was found in samples acidified with HCl if evaporation at high temperatures (eg 87°C) was prolonged until a NH_4Cl film formed. Samples acidified with H_2SO_4 were significantly contaminated with atmospheric ammonia when the evaporation procedure was prolonged. The samples acidified with boric acid produced erratic ^{15}N values which may be due to incomplete breaking of the bonds in $(NH_4)_3BO_3$.

2.3 Collection and Preservation

In a typical sample collection of N-containing substances in the field, a large quantity of material is gathered and is preserved according to it's nature. If the material consists of living organisms, then it is sorted (as best as possible) into different species in the field, then placed in a plastic bag and stored in ice (in a chilly bin) to reduce respiration to a minimum. Other substances such as sediments and liquids are collected in 1L plastic jars, the sediments receive no special treatment in the field, however liquids are immediately acidified (to pH 4-6) to stabilise NH_4-N in the solution and stored in ice. Upon returning from a field excursion, samples are either stored overnight in a 0-4°C cold room or preserved (in the case of living organisms and sediments) or processed immediately (in the case of liquids).

Preservation of living organisms and sediments is preceded by the removal of unwanted substances (eg soil from around plants) and washing in reverse osmosis water (RO water)before oven drying in a 60°C forced air oven. Organism are subsamped for identification of species before drying. All sample material was dried in a Contherm series 5 oven for one to two days, before grinding.

Dried material was ground using a mortar and pestal, then redried before storing in a desiccator. The material was ground until particles of no larger than 5mm diameter remained and redried for 15 minutes before placing in a vacuum sealed desiccator and stored until required for N analysis.

2.4 Mass Spectrometer Analysis

2.4.1 Introduction to Mass Spectrometry

The Mass Spectrometer (M. S.) is a machine that is used for measuring relative abundance of isotopes in a substance by comparison with a known standard isotopic material. The M. S. ionizes molecules in the gas phase by bombardment of the molecules with an electron stream emmitted from a hot Tungsten filament, this knocks out electrons leaving charged residuals behind. The M. S. sorts the ions produced into a spectrum, depending on their ratio of mass to charge (ie M / e), which is achieved using a range of electrical and magnetic fields. For nitrogen analyses, the ions produced are : $^{14}N^{+1}$; $^{14}N^{14}N^{+1}$; $^{14}N^{14}N^{+2}$; $^{14}N^{15}N^{+1}$; $^{14}N^{15}N^{+2}$; $^{15}N^{15}N^{+1}$ and $^{15}N^{15}N^{+2}$. The M. S. collects the $^{14}N^{15}N$ and $^{14}N^{14}N$ forms (some machines also collect the $^{15}N^{15}N$ form, eg The Tracer M. S. used in this study) on insulated electrodes, and the resulting current produced from the ions are measured to determine the relative ^{15}N abundance of the sample. The ionic forms not collected, occur only at very low frequency and contribute only a small error to the overall calculation of ^{15}N abundance.

2.4.2 Micromass 602E

2.4.2.1 Introduction

The Micromass 602E M. S. is a dual inlet, dual collector mass spectrometer, and is automated to provide a measurement of ^{15}N abundance, which is based on ten consecutive resolutions of the difference in the ratio of mass 29 / mass 28 of a sample to a reference (Nevins et al, 1985). The Micromass 602E is able to accurately estimate the ^{15}N abundance of the sample to within

0.025$^{o}/_{oo}$, by measuring alternately the sample and reference gases. Some imprecision is introduced in the switching from the reference to the sample, which results in a small degree of mixing and accounting for a significant part of the error produced.

To be able to use the Micromass spectrometer 602E to measure the ^{15}N abundance of N in a sample, both the sample and the reference must be in the N_2 form. The reference used is cylinder N_2 gas, therefore it is only necessary to convert the various N - compounds in the sample to N_2 gas. This is carried out by converting the various nitrogen forms found in the sample to firstly ammonium salt, using the Kjeldahl process, and then to N_2 gas on a vacuumline.

2.4.2.2 Kjeldahl digestion

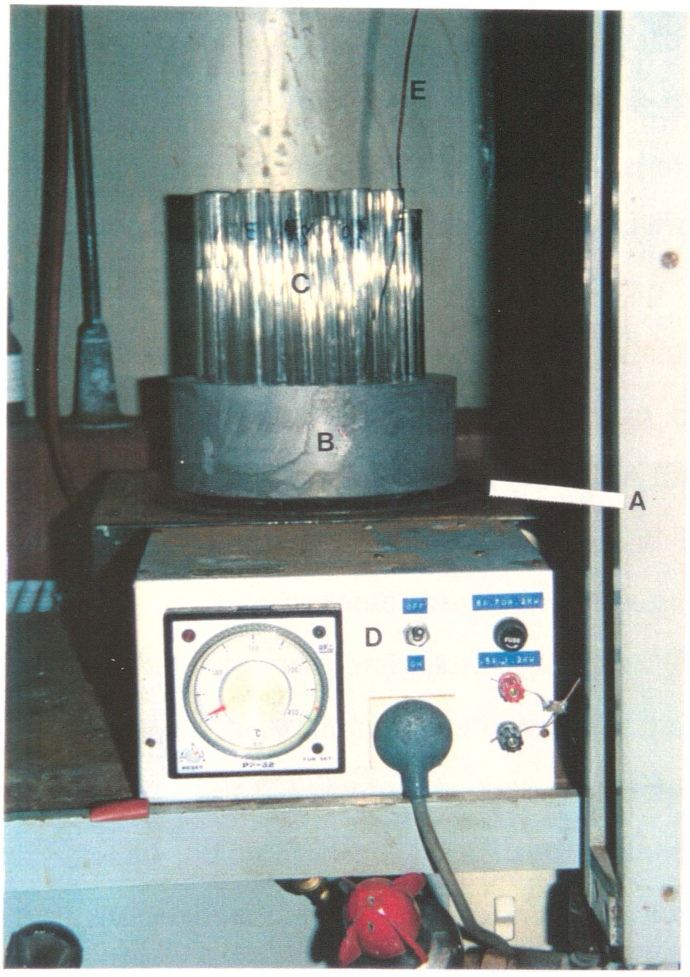
N - containing substances that have been oven dried are ready to be digested to extract tissue nitrogen, this process is carried out using the Kjeldahl method. In the Kjeldahl process samples of nitrogenous substances are digested by concentrated acids at high temperature to release nitrogen from the compounds in the material and convert it into a stable ammonium salt.

Plate 2.1 shows the equipment used in the Kjeldahl digestion procedure. The main structural features are a cast aluminium block with holes drilled in it to hold Kjeldahl tubes and two temperature controls (a simmerstat connected in series to a proportional controller that operates through a thermocouple linked to the block).

In using the Kjeldahl method, the aluminium block is preheated to 200°C by adjusting the proportional controller (the simmerstat is set on high and is not changed), while subsamples of the materials to be analysised are accurately weighed out (need ca.150 mg) and transferred to a Kjeldahl tube. During the

weighing and transferral to the tubes it is important that care is taken to ensure that no material is trapped on the sides of the tubes and that any sample that is not transferred is not added to the weight. Digestion mixture (Appendix B1) is now added, 5mls for plant material and 10mls for sediments (and two to three boiling chips per tube). The Kjeldahl tubes are now ready to be placed in the preheated block (200 °C) and digested.

During the early stages of the digestion process carbohydrate will char (Kjeldahl solution turn black) and all the water will boil off, however sometimes a crust can form and this will need to be knocked back into the solution. When white smoke appears in all of the Kjeldahl tubes, then the proportional controller can be turned up to 360 °C and samples left to digest for ca. 2 hours. During this time at 360 °C the colour of the Kjeldahl solution will change from black to black / red to green or yellow, when either the green (or yellow) colour is reached, this indicates that all the N is now in an ammonium salt form (NO_3 and NO_2 are not reduced to NH_4 in this method described), and the Kjeldahl tubes can be removed from the heat, allowed to cool. At this stage the Kjeldahl tubes may be stored until required for NH_3 distillation.



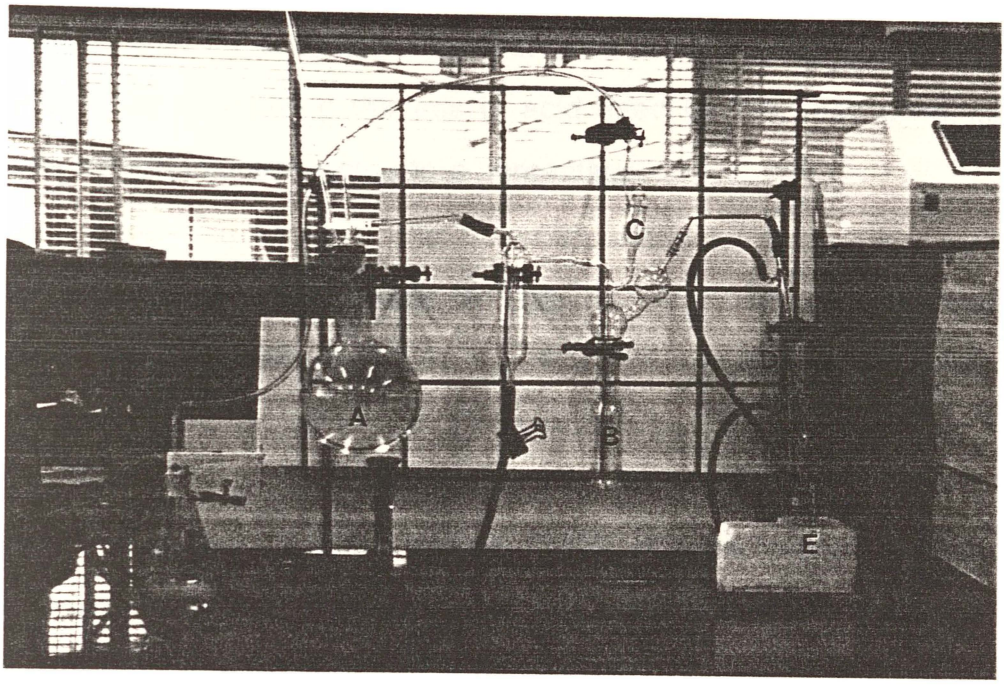
2.1 : Kjeldahl digestion block. Consisting of a hotplate fitted with a simmerstat (A). The aluminium block (B) has drilled holes to fit standard test tubes (C) containing the sample material and digestion mixture. A proportional controller (D) regulates the temperature via a thermocouple (E).

2.4.2.3 Steam Distillation and Titration

After Kjeldahl digestion of plant (or sediment) material, nitrogen can be extracted from the samples by increasing the pH using NaOH, to >10 (coverts all the ammonium salt to ammonia), steam distilling, and then calculating the total quantity of N by back titrating the distillate against a standard acid. In order to steam distill digested samples a Panas Wagner still (Plate 2.2) is used in conjunction with a silver condenser to distill and trap the ammonia produced. Steam is generated in (A) a round bottom flask (containing distilled water and glass balls) by heating with a bunsen, a water trap, collects water vapour produced, and the hot air is forced through a narrow tube into a still (B). The Panas Wagner Still contains the digested sample and 10mls of 10M NaOH (appendix B2). The hot air circulates the NaOH through the sample, thus raising the pH to >10 and the temperature, this ensures that all NH_4 is converted to the volatile NH_3 form. Ammonia volatilises from the solution and is collected in the silver condenser (C) where it is cooled collected in a 150 ml flask (D) containing a mixed indicator (Methyl Red and Bromocresol Green) solution (appendix B3). Approximately 70mls is collected in the flask before the process is stopped and the distillate is ready to be titrated.

Liquid samples that comprise solutions of NO_3 and / or NH_4 are distilled on the Buchii still in preference to the Panas Wagner, due to the Buchii's ability to handle larger sample volumes and cater for the extra step involved in NO_3 extraction from a solution. The Buchii uses a glass condenser and has a removable still that is able to handle sample volumes of up to 250 mls. When using the Buchii NH_3 is distilled off first, then NO_3 is converted to ammonia by the addition of Devarda's Alloy, which acts as a H^+ donor in the reaction , NH_4 produced is then distilled as per normal.

To determine the nitrogen content of a sample the distillate is titrated against a standard HCl acid solution (either .1N or .01N) on a Multi Dosimat E 415 type automatic titrator, until the grey endpoint colour of the mixed indicator



2.2 : Ammonia distillation apparatus. Steam is generated in the round bottom flask (A) and passes into a Panas Wagner Still (B) containing the sample material and NaOH added via a entry spout (C). The NaOH raises the pH, the steam enhances ammonia volatilisation. Ammonia and water vapour is cooled in a silver condensor (D) and then collected as water droplets in a 150 ml Erlmeyer flask (E) containing a mixed indicator solution.

is reached. After titration, the nitrogen content can be determined by using one of the following equations (eq. 2.1 and 2.2), the equation used depends on the concentration of the HCl.

When titrating a distillate against 0.1N HCl the percentage nitrogen is calculated by multiplying the mls of acid used by 1.4, then dividing by the sample weight and converting to a percentage.

$$\% N = \frac{1.4 \times (\text{mls of .1N HCl used})}{\text{sample weight (mg)}} \times \frac{100}{1} \qquad \text{(eq. 2.1)}$$

When titrating a distillate against 0.01N HCl the percentage nitrogen is calculated by multiplying the mls of acid used by 1.4, then dividing by the sample weight and converting to a percentage.

$$\% N = \frac{0.14 \times \text{mls of .01N HCl used}}{\text{sample weight (mg)}} \times \frac{100}{1} \qquad \text{(eq. 2.2)}$$

2.4.2.4 Evaporation

After distilling and titrating the N - samples, the solution is evaporated until only the ammonium salt crystals remain using two steps. In the first step the samples are transferred to 50 ml flasks and evaporated on a hotplate at just under boiling temperature (ie. 90 - 95°C) until about half a millilitre of sample remains. In the second step the remaining solution is removed from the heat, transferred to a quickfit testtube and crystallised in a 60°C, Contherm series 5 oven. This step takes approximately seven days, once this is completed they are ready to be run on the vacuumline.

2.4.2.5 Vacuumline Procedure

The vacuumline apparatus is used to convert NH_4Cl crystals left to N_2 gas (the form of nitrogen measured by the Micromass 602E mass spectrometer). The vacuum line used is a dual sided line featuring a toepler pump and two cold traps per line. In the vacuumline N conversion process (fig. 2.1), LiOHBr is added to (A) the testtube containing the NH_4Cl sample and the resulting reaction releases nitrogen and bromine gases, Br and water vapour is collected in (B) the first cold trap, with the N_2 gas passing straight through and into (C) the toepler pump which is then sealed. The second half of the vacuumline is now opened and the N_2 gas is forced out of the toepler pump through (D) the second cold trap and is collected, cooled and sealed into (E) a glass tube. The glass tube containing N_2 gas is now ready to be run on the Micromass 602E mass spectrometer.

2.4.2.6 ^{15}N Analysis

The ^{15}N abundance of a sample N_2 gas (collected on the vacuumline), can be determined by using the Micromass 602E mass spectrometer (M. S.). The glass tube containing the sample is inserted into the sample inlet, and the sample pressure balanced with the reference gas. When the sample and reference pressures are the same, the mass spectrometer analysis can begin. The mass spectrometer starts on the reference side, the N_2 gas is ionised, and the two positive ion beams collected ($^{14}N^{15}N$ and $^{14}N^{14}N$) are measured simultaneously by the dual electron-capture detectors. Following this the M. S. automatically switches to the sample side and repeats the same processes. Once an analysis has begun the M. S. will continue to oscillate between the reference and sample sides, until the required number of comparisons is made, and then it calculates the ^{15}N abundance in the sample.

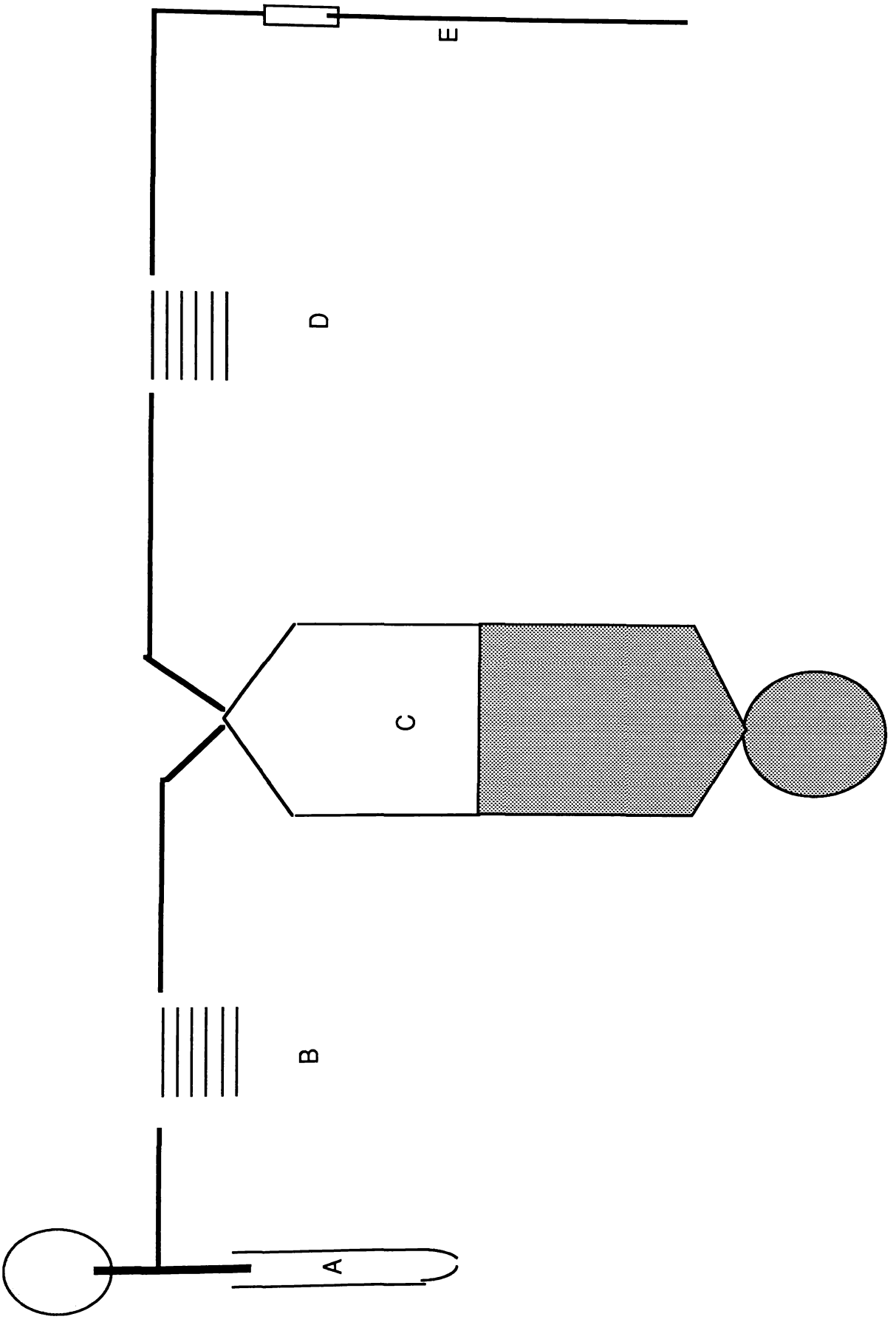

E
D
C
B
A

Figure 2.1 : Schematic diagram of the Vacuumline apparatus used for conversion of NH_4Cl to N_2. LiOHBr is added to the NH_4Cl (A), Br and water vapour is collected in the first cold trap (B) with the N_2 gas collected in the toepler pump (C). The nitrogen is forced out of the pump through a second cold trap (D) and is collected in a glass tube (E).

The normal operating procedure on the Micromass 602E mass spectrometer is to run two or three standards (NH_4Cl), at the beginning, to check that the machine is running satisfactory, before running samples. The operating procedure for the Micromass 602E, requires that the previous standard (or sample) is evacuated from the machine before the next one is run. The run takes approximately 10 minutes for the M. S. to analysis the sample and calculate it's ^{15}N abundance.

2.4.3 Europa Tracermass

2.4.3.1 Introduction

The Europa Tracermass spectrometer is a single inlet, triple collector mass spectrometer, and is automated to provide a measurement of ^{15}N abundance, based on the ratio of mass 30 / mass 29 / mass 28 of sample compared with the ^{15}N abundance of a known reference (run previously). The Tracermass is able to accurately estimate the ^{15}N abundance of the sample to within $0.1^{o}/_{oo}$, by measuring alternately the sample and reference. Some imprecision is introduced into the ^{15}N abundance estimation by the M. S. due to residual N being left in the single inlet, which is used for both reference and sample materials, resulting in a small amount of mixing between the various substances.

To be able to use the Tracermass spectrometer to measure the ^{15}N abundance of N in a sample, both the sample and the reference must be in the N_2 form. The reference and sample materials are changed to N_2 gas using a Carlo Erba elemental analysiser.

2.4.3.2 Preliminary Preparation

In order to run samples on the Tracermass spectrometer, the materials need to be in a solid state and packaged in tin cups, before they are ready for analysis. Samples that are already solid (eg. dried, ground plant material and sediments) usually will not need to have their present state altered, however if the N content of a material is very low (ie. below 0.1%) or the substance is a liquid then they will have to be converted to an ammonium salt . The materials are weighed into small tin cups (require ca. 1 mg N) which arepressed into a spherical shape and placed in a sample tray for M. S. analysis.

2.4.3.3 Combustion

The Carlo Erba NA 1500 Elemental Analyser, is a machine that can be used to separate C, N and S from various solid materials to enable the measurement of their respective stable isotopes by a M. S., however as only the N isotopes are of interest in this study, the process with respect to N will be discussed. Samples are placed in a carousel attached to the Carlo Erba. The method of loading the carousel, is to place two standards (Urea) first, then twelve samples, two more standards, twelve samples, and so on, up to a maximum of 120 samples. The first standard is used later in the M. S. analysis as a check of accuracy, and the second is used to recalibrate the machine. Once loaded analysis can be initiated.

Once the combustion process is initiated the carousel automatically releases samples at set intervals into the Carlo Erba, where the material is combusted in an oxidation column. The combusted sample releases various gases, which pass through carbon dioxide and water filters (to remove impurities) before entering a copper reduction column. In this column, the various oxides of nitrogen are reduced to N_2, which then pass into a gas

chromatograph. The gas chromatograph separates the molecules of N_2 (from the other molecules) and then transfers the N to a mass spectrometer for analysis of ^{15}N abundance.

2.4.3.4 ^{15}N Analysis

The Tracermass ionises the N_2 gas, and the $^{14}N^{15}N$, $^{14}N^{14}N$ and $^{15}N^{15}N$ ion beams produced are then measured simultaneously by the triple electron-capture detectors. The M. S. will calculate the area under the curves produced by the ion beams and determine the ^{15}N abundance, and calculate the percentage nitrogen combustion of the substance being analysised.

2.5 Statistical Calculations

In expressing experimental data two main statistical calculations (mean and standard error) have been used to best show the N concentration and N isotope abundances . The mean is the average of three replicates (eq. 2.3). Equation 2.3 shows that the mean can be calculated by adding either the N concentration or isotope abundance values for the replicate samples together and dividing by N (the number of subsamples).

$$\bar{X} = \frac{X_1 + X_2 + \ldots + X_n}{N} \qquad \text{(eq. 2.3)}$$

The Standard Error (SE) is a estimation of the 95 % Confidence Interval (CI) for the variation in the N content and isotope abundance values and can be calculated by using eq. 2.4. Equation 2.4 shows that the SE is equal to 1.96 (the multiplier value for the 95 % CI) times (S_{n-1}), the standard deviation of a

small population, divided by the square root of (N), the number of replicate samples.

$$SE = \frac{1.96\, S_{n-1}}{\sqrt{N}} \qquad \text{(eq. 2.4)}$$

2.6 Method Tests

2.6.1 Introduction

The various methods used to prepare samples for Micro and Tracer mass spectrometer analysis were tested for nitrogen recovery and / or effect on the estimate of ^{15}N abundance in a sample. Firstly the Kjeldahl method (Section 2.4.2.2) was compared with the Combustion method (Section 2.4.3.3) for efficiency in converting N - compounds in various materials to either NH_4 - N (Kjeldahl method) or N_2 (Combustion method). Secondly the distillation process (Section 2.4.2.3), was assessed for the ability to recover NH_4 - N and NO_3 - N from a standard NH_4NO_3 solution. Thirdly the Evaporation method (Section 2.4.2.4) used in preparing samples for Micromass spectrometer analysis was assessed for it's effect on the M. S. estimate of ^{15}N abundance. Fourthly a comparison of the Micromass and Tracermass spectrometer estimates of ^{15}N abundance in two different plant samples was carried out. Finally an estimation of the isotope ratio for the expected major source of N contamination during sample preparation was carried out.

2.6.2 Recovery of N

2.6.2.1 Kjeldahl vs Combustion

The Kjeldahl and Combustion methods (Sections 2.4.2.2 and 2.4.3.3), used for nitrogen determination, were compared for nitrogen recovery from sediment and from various plant samples. Three replicates of a sediment sample and four different plant species were run through the Kjeldahl digestion procedure, with another set of replicates (of the sediment sample and each plant species), under going the combustion method for nitrogen

determination. The amount of nitrogen recovered from each of the methods (Kjeldahl and Combustion) were compared.

Table 2.1 shows the percentage of nitrogen in Manukau sediment, Gracillaria, Ulva, Mangrove and Lagarosiphon as determined by the Kjeldahl method (Section 2.4.2.2) and the Combustion procedure (Section 2.4.3.3). The values obtained for nitrogen content by the Kjeldahl and Combustion methods are used as a comparison between the methods.

Sample	Kjeldahl (Kjel.) %N	Combustion (Com.) %N	Kjel. vs Com. % Com.
Sediment	0.102 ± 0.022	0.103 ± 0.010	99
Gracillaria	5.65 ± 0.15	6.22 ± 0.36	91
Ulva	4.43 ± 0.03	4.65 ± 0.10	95
Mangrove	2.37 ± 0.02	2.53 ± 0.08	94
Lagarosiphon	2.48 ± 0.14	2.72 ± 0.15	91

Table 2.1: Results from the determination of nitrogen content by the Kjeldahl (Kjel.) (Section 2.4.2.2) and Combustion (Com.) (Section 2.4.3.3) methods, for Manukau Harbour sediment and Gracillaria, Ulva, mangrove and Lagarosiphon plant samples. The comparison between the two methods is shown by Kjeldahl - N as a percentage of Combustion - N.

2.6.2.2 Distillation

The distillation method used to extract NH_4 - N (Section 2.4.2.3) and NO_3 - N (Section 2.4.2.3) was tested for the ability to recover nitrogen from a standard solution of ammonium nitrate. The standard solution of ammonium nitrate contains 0.2g of NH_4 - N and 0.2g of NO_3 - N in distilled water. Three, 10ml samples of ammonium nitrate and a blank of distilled water were run on the Buchii still using the previously discribed methods (Section 2.4.2.3), to test the ability of the still in extracting NH_4 - N and NO_3 - N from a solution.

Table 2.2 shows the titrations for a blank (distilled water) and standard NH_4NO_3 solution (average of three replicates). The milligrams of NH_4 - N and NO_3 - N is obtained by using equation 2.1, with the percentage of NH_4 - N and NO_3 - N recovered calculated by comparing with the expected values of 0.2g l^{-1} NH_4 - N and 0.2 g l^{-1} NO_3 - N.

Sample	Titration 0.1N HCl (mls)	Nitrogen NH_4 - N (mg)	Recovery NH_4 - N (%N total)	Titration 0.1N HCl (mls)	Nitrogen NO_3 - N (mg)	Recovery NO_3 - N (%N total)
Blank (d. H_20)	0.02	0.0	-	0.03	0.0	-
Average (3samples) (NH_4NO_3)	1.43 ± .01	1.979±.01	98.9 ± 0.5	1.44 ± .02	1.969±.02	98.5 ±1.2

Table 2.2: Ammonium - N and Nitrate - N titrations after Steam distillation of a blank (distilled water) and standard NH_4NO_3 solution (three replicates). The percentage recovery of NH_4 - N and NO_3 - N by the method is also shown.

2.6.3 ^{15}N Abundance Estimates

2.6.3.1 Evaporation

The reliability of the evaporation method (Section 2.4.2.4) to maintain the ^{15}N abundance of NH_4Cl during the transformation from an aqueous solution to crystals was examined by assessing the ^{15}N abundance of a solution of NH_4Cl with a known ^{15}N abundance that was crystalised using this procedure. The ^{15}N abundance of AR grade NH_4Cl was determined by mass spectrometry and compared with the natural abundance of recrystalised AR grade NH_4Cl, with the results of three replicate analyses shown in table 2.3.

Sample	Atom % ^{15}N	^{15}N
Recrystalised NH_4Cl	.36651 ± .00008	0.57 ± 0.17
Shelf NH_4Cl crystals	.36636 ± .00008	0.16 ± 0.17

Table 2.3 : The natural abundance of AR grade NH_4Cl, and AR grade NH_4Cl after recrystalisation by the evaporation method (Section 2.4.2.4).

2.6.3.2 Mass Spectrometry

The overall Micromass and Tracermass spectrometer methods for estimating ^{15}N abundance in N - containing materials was assessed for two plant samples. Two different plant samples (A and B) were processed by both the Micromass and Tracermass preparation methods and run on the respective mass spectrometer to obtain an estimate of their ^{15}N abundance.

Table 2.4 shows the estimate of ^{15}N abundance in two plant samples (A and B), using the Micromass and Tracermass spectrometers. The estimates of ^{15}N abundance in samples A and B are shown as the average of three replicates.

Sample	Micromass analysis Atom % ^{15}N	Tracermass analysis Atom % ^{15}N
A	.36567 ± .00009	.36598 ± .00022
B	.36682 ± .00026	.36738 ± .00030

Table 2.4 : Estimates of ^{15}N abundance in two plant samples: (A) *Lagarosiphon major* and (B) *Enteromorpha nana* using the Micromass method (Section 2.4.2) and the Tracermass procedure (Section 2.4.3).

2.6.4 N contamination

The Micromass and Tracermass ^{15}N analysis methods allow for the introduction of N contamination at various stages during the sample preparation process. Ammonium and nitrate may be added to samples during the distillation process (Section 2.4.2.3) where a small amount of distilled water is condensed from the steam that heats up the still. Further more during the evaporation process (Section 2.4.2.4) atmospheric ammonia may be trapped in the acidified solutions. Finally in the packaging of samples for Tracermass spectrometer analysis (Section 2.4.3.2) N (ie. amino acids) may be transferred to the tin cups from contact with skin. However all these potential sources of errors mentioned (except packaging contamination) would be expected to result in a relatively small, consistent addition of N (isotopic composition at approximately natural abundance), with distilled water being the most likely source of contamination. Packaging may add considerably more N than other sources, although careful handling will negate this error. A study of the concentration and N isotope abundances of NH_4 and NO_3 - N in tap water was carried out to determine the potential effect of distilled water containination.

In order to examine the ^{15}N abundance of NH_4 and NO_3 - N in tap water, three 20 l replicates were concentrated by evaporation (Section 2.4.2.4) to 250mls each, and prepared for mass spectrometer analysis by the Tracer mass method (Section 2.4.3.2). The results, for the Tracermass spectrometer ^{15}N analysis of tap water NH_4 and NO_3 - N is shown in Table 2.5.

The total concentration of dissolved inorganic nitrogen in tap water was 95 ± 6 ug l^{-1} with .36581 ± .00030 Atom % ^{15}N. NH_4 - N proportion of the total dissolved - N was found to be 38 ± 4 ug l^{-1} with .36519 ± .00011 Atom % ^{15}N, while NO_3 - N made up 57 ± 7 ug l^{-1} with .36622 ± .00042 Atom % ^{15}N.

N - form	Concentration ($ug\ l^{-1}$)	Atom % ^{15}N	$d^{15}N$
NH_4	38 ± 4	.36519 ± .00011	-3.0 ± 0.3
NO_3	57 ± 7	.36622 ± .00042	-0.2 ± 1.1
$NH_4 + NO_3$	95 ± 6	.36581 ± .00030	-1.3 ± 0.8

Table 2.5: Average concentration and isotopic composition of NH_4 - N and NO_3 - N in three 20 l samples of tap water.

Chapter 3

Study Introduction

3.1 Preamble

Stable isotope methodology is useful in natural abundance tracer studies only when certain conditions for their application are met. Firstly there needs to be a source of nitrogen which is significantly different in it's ^{15}N abundance to the receiving waters (Section 1.4.3). Secondly the source nitrogen should not be significantly discriminated against during biological processes or at worst the size of the isotope effect should be known. Lastly in order to gain qualitative and quantitative data it is necessary that information is available (or is collected) regarding water flow rates and the contributions of various N-sources to the total N-flow.

The first condition for application of the stable isotope method to wastewater studies has been recently shown in preliminary studies on the natural abundance of sewage NH_4-N (Halley, 1990 and Miller, 1990). The effluent studies were carried out on treated sewage from Horotiu (Halley, 1990) and Taupo (Miller, 1990), and have been examined as part of this project, along with effluents from a variety of treatment stations (Chapter 4). The mechanism that results in ^{15}N enrichment of sewage NH_4-N, has been investigated by a preliminary study into process effects on ^{15}N abundance in the Horotiu treatment system (Section 4.3).

The second condition, the problem of discrimination in using the stable isotope method for waste water tracing has been met by using direct measurements of ^{15}N abundance when ammonium concentration is high (eg Sewage ponds) and indirect measurements using *insitu* plants when the ammonium concentration is low (eg Receiving waters). Chapter 4 shows the

results of a study of the ammonium isotope ratio (^{15}N / ^{14}N) in the sewage plants and chapter 5 contains the values (from plants) for the Upper Waikato River and the Northern Manukau Harbour.

The last condition concerning flow rates, both of water and nitrogen has been obtained by using previously gathered data from the study of Huser (1989) to produce a model of the Upper Waikato River and it's uptake system (Chapter 6). Similar data for the Northern Manukau Harbour (Vant, 1991) also allows for a preliminary model of that system to be produced (Chapter 6).

3.2 Sewage Treatment

In New Zealand raw sewage is usually processed at a treatment plant that maintains processes under non-equilibrium conditions. The treatment system utilises biological reactions (eg nitrification and assimilation) kept in the logarithmic phase of the growth curve by continuous addition and removal of substrate and products and physical processes (eg gas diffusion) that are usually reliant on outside factors (eg Wind speed or electricity) to maintain them. Raw sewage is typically high in BOD and disease organisms, the removal of these constituents are a prime target of treatment processes which often also reduce the solids and nutrients (especially nitrogen and phosphorous) as well. Hence as this current study is concerned with stable isotope ratios (in particular nitrogen) discrimination between the nitrogen isotopes, during processing can be discovered by examining the natural abundance of ^{15}N in NH_4-N (the major form of dissolved nitrogen) in the various systems within a treatment plant.

The systems chosen to treat sewage are constrained by several factors such as finance, space, soil type and the constituents of the waste water. However each treatment plant aims to carry out a similar function in removing nutrients and particles from the sewage. Although in general there is a trade

off between the space available and cost, which dictates the mechanisation used, the most popular method of treatment is oxidation ponds (Table 3.2), which are relatively cheap to build and maintain but require a sizeable land area. Similarly wetland treatment systems are cheap to build and operate but occupy a large space. At the other end of the spectrum, compact, mechanised systems are more costly to run, however their level of throughput is much higher than the equivalent pond system.

Three levels of sewage treatment are currently carried out in New Zealand: primary, secondary and tertiary (Manning, 1991). In primary treatment the bulky and easily separated solids are removed, by skimming the fats off the top and scraping the sludge from the bottom and then treating them separately, with the products often finishing up as composts or soil conditioners. Secondary treatment of sewage effluent normally consists of settlement of suspended solids and aeration of liquids, with the settled solid undergo natural anaerobic fermentation while the suspended and soluble materials are variously aerated to stimulate aerobic reactions. The aerobic reactions with the suspended and soluble materials are facilitated either by recirculation over solid plastic or concrete media, or by natural diffusion and photosynthesis reactions in large ponds. The final treatment level, tertiary (also known as polishing) involves the removal of the dead microbe cells and the reduction of the effluent nutrient levels to minimise the effect of a high oxygen demand and rapid plant growth in the receiving environment. The end result from a sewage treatment plant is the production of effluent with reduced pollutant levels that will have a lower impact in the environment than raw effluent.

A recent survey (Shields, 1991) outlines current sewage treatment procedures undertaken by local authorities in New Zealand with populations under 20 000 (total population covered is 2.5 million people), covering levels of treatment, treatment systems and receiving bodies. The first category surveyed, level of sewage treatment (Table 3.1) revealed that all sewage

receives at least primary treatment with 59% of the treatment plants carrying out secondary but only 9% tertiary treatment. The second category surveyed, method of sewage treatment (Table 3.2) indicates the systems used by the various authorities. The survey showed that oxidation ponds are by far the most common system used (used in 40% of all systems cf. the next most common, millscreening 10%). The high use of oxidation ponds reflects the amount of space available on a treatment plant site and the relatively low cost of setup and maintenance of each system. The last category surveyed was the receiving body used for the treated effluent , this is presented in table 3.3, with related receiving bodies grouped together (eg River and Estuary). Table 3.3 shows that all the receiving bodies used are in someway linked with some sort of water body (except land), indicating perhaps the geographical features and the locations of settlements in New Zealand. Most urban developments (big and small) in New Zealand are sited on the coast (eg Auckland, Greymouth), by a lake (eg Rotorua, Queenstown) or on the banks of a river (eg Hamilton, Roxburgh).

3.3 Introduction to Major Study areas

In assessing the potential of using ^{15}N abundance as a tracer of sewage in this study it is necessary that we review the major sites used in some detail with respect to the nature of the effluent treated, systems involved and features of the respective receiving bodies (if relevant). In this current ^{15}N tracer study, the preliminary work was focused on the effluent from the Horotiu and Upper Waikato River dissolved ammonium, with later work carried out in the Manukau Purification Works and Manukau Harbour.

The first major experimental system used in this study was the Horotiu treatment plant located ca. 13 km north of Hamilton along state highway one and situated on the western bank of the Waikato River (fig. 3.1), which

processes sewage produced from the nearby Affcco freezing works. The effluent produced at Horotiu has been previously shown to be enriched in ^{15}N (Halley, 1990) and therefore is a good system to investigate, due to it's close proximity to Hamilton and it's previously proven isotopically enriched nature. In the Horotiu treatment procedure (fig. 3.2), effluent passes through primary treatment where fats are skimmed off the top and sludge is scraped from the bottom of the tank. The effluent from primary treatment is stored in an anaerobic balance pond and is distributed to a second anaerobic pond at a constant rate. In the second pond anaerobic organisms digest the suspended and dissolved nutrients in the sewage. The digested sewage in the anaerobic pond is transferred to a clarifier pond, which is aerated to create an aerobic environment that will kill the anaerobes. The anaerobic reaction is stopped which also results in a high carbon loss. The effluent from the clarifier pond passes into an facultative pond where further deposition of suspended particles and the volatilisation of ammonia can occur. Effluent from the oxidation pond is finally discharged into the Waikato River. Two experimental systems also receive a small proportion of the effluent flow through them: the wetlands receive sewage from the clarifiers and deposit it into the oxidation pond and the forestry block receives effluent from the oxidation pond at a set rate and frequency. This study at Horotiu was not continued in the receiving body (ie Waikato River) so the local features of the river won't be described here.

The second major system that has been used in this study is the Taupo Borough sewage discharge into the Waikato River (fig. 3.1). The Waikato River drains New Zealand's largest lake, Lake Taupo which itself is the crater of a large caldera type volcano. Hence geothermal activity is significant beneath the lake and the Upper reaches of the river. The Waikato river has high aesthetic values, being used as a recreational area and tourist site as well as being an important agricultural, industrial and domestic receiving body for both point and diffuse effluent discharges from activities within it's catchment area.

The water in the Waikato River becomes increasingly enriched as it flows from the Taupo Gates to it's final outflow at the Port Waikato (Neilson, 1979). Neilson showed that a considerable part of the nutrient load to the river was due to geothermal and agricultural activity in the upper reaches of the Waikato river between the Taupo Gates and Lake Ohakuri, while also noting that that the effluent soon to be discharged from the Taupo Borough Plant would cause a significant future impact in the Upper Waikato River (defined as the area between the Taupo Gates and Lake Aratiatia, fig. 3.3). Recent monitoring of the Upper Waikato River by Huser (1989) has shown that there is a significant increase in ammonium immediately below the treatment plant outlet to at least three times the upstream concentration (ie >14 mg m^3 downstream cf. 4 mg m^3 upstream), which was attributed to the impact of the effluent discharge. The data produced by Huser (1989) shows that ca. 60% of the total ammonium in the Upper Waikato River is effluent sourced (that is if we ignore the diffuse source groundwater). Stable isotope methodology has been applied to this system in an attempt to identify the impact of effluent ammonium in the river.

The final system examined in this current study, the Manukau Purification Works discharge into the Manukau Harbour (fig. 3.4). The Manukau Plant (fig. 3.4) in South Auckland treats predominantly Auckland domestic sewage to secondary level (Section 3.2). Incoming raw sewage is digested and separated into sludge and liquid effluent during primary treatment, with the sludge transferred to a lagoon (Lagoon 1-4, fig.3.5), while the bulk of the liquid effluent goes through a filtration system before being carried via a channel to aerobic pond 1 (fig. 3.5) and is then circulated through ponds 2,3 and 4 (in that order), before it is discharged into the harbour.

In the Manukau Harbour the northern most third is the region which receives the effluent from the Mangere plant, this region is referred to as the Northern Manukau Harbour. In the Northern Manukau Harbour effluent that has been released on an out going tide travels down the Purakau Channel (fig 3.4) and accumulates in the lower reaches of the channel (fig. 3.4) before

being recirculated up the channel on the in coming tide, past the sewage works and down the Wairopa Channel and finally being carried out the harbour mouth. Hence the most noticeable effects from the effluent (eg prolific growth of the high nutrient indicator Gracillaria) are seen in the Northern Manukau Harbour. In this current study various sites in the harbour have been visited and samples of organisms and sediments have been gathered along with effluent and algae from the Manukau Purification Works.

Treatment Level	Number of Systems	Percentage of Total (%)
Primary	74	100
Secondary	44	59
Tertiary	7	9

Table 3.1 : Sewage Processing in New Zealand. The number of systems that carry out sewage treatment at primary, secondary and tertiary levels. Results from a survey of 42 local authorities servicing a population of 2.5 million people (adapted from Shields, 1991).

Treatment System	Number of Systems	Percentage of Total (%)
Oxidation Pond	49	40
Milliscreening	12	10
Filtration	9	7
Sludge Digestors	7	5.5
Wetlands	7	5.5
Other (12 systems)	40	32

Table 3.2 : Most common treatment methods of sewage processing in New Zealand. Results from a survey of 42 local authorities servicing a population of 2.5 million people (adapted from Shields, 1991).

Receiving Body	Number of Systems	Percentage of total (%)
River / Estuary	33	51
Sea / Harbour	21	32
Land	10	15
Lake	1	2
Unspecified	11	-

Table 3.3 : Receiving waters for sewage in New Zealand. Results from a survey of 42 local authorities servicing a population of 2.5 million people (adapted from Shields, 1991).

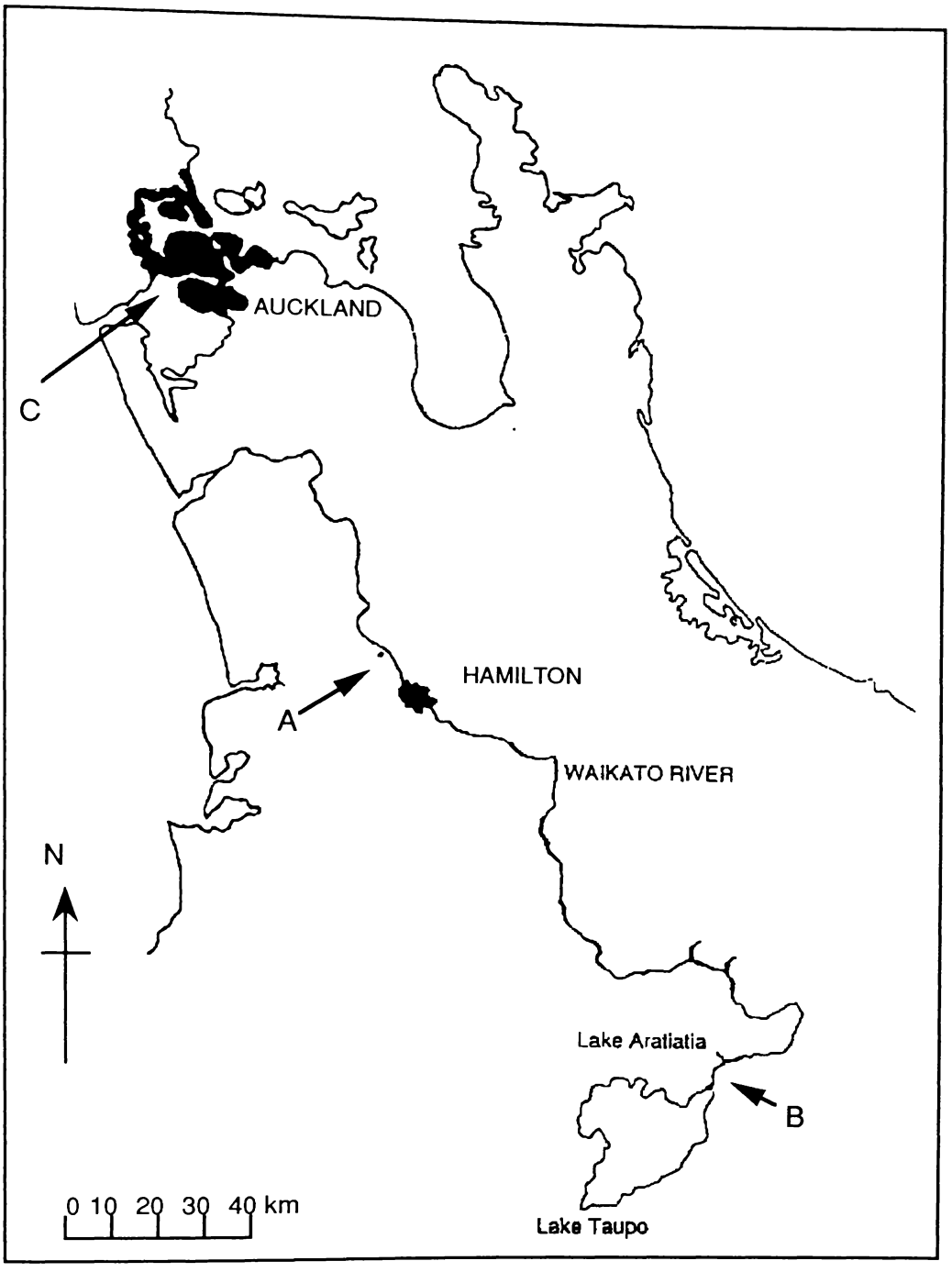


Figure 3.1 : Map showing the location of the Horotiu treatment plant (A), Upper Waikato River (B) and Northern Manukau Harbour (C) study sites in the North Island of New Zealand.

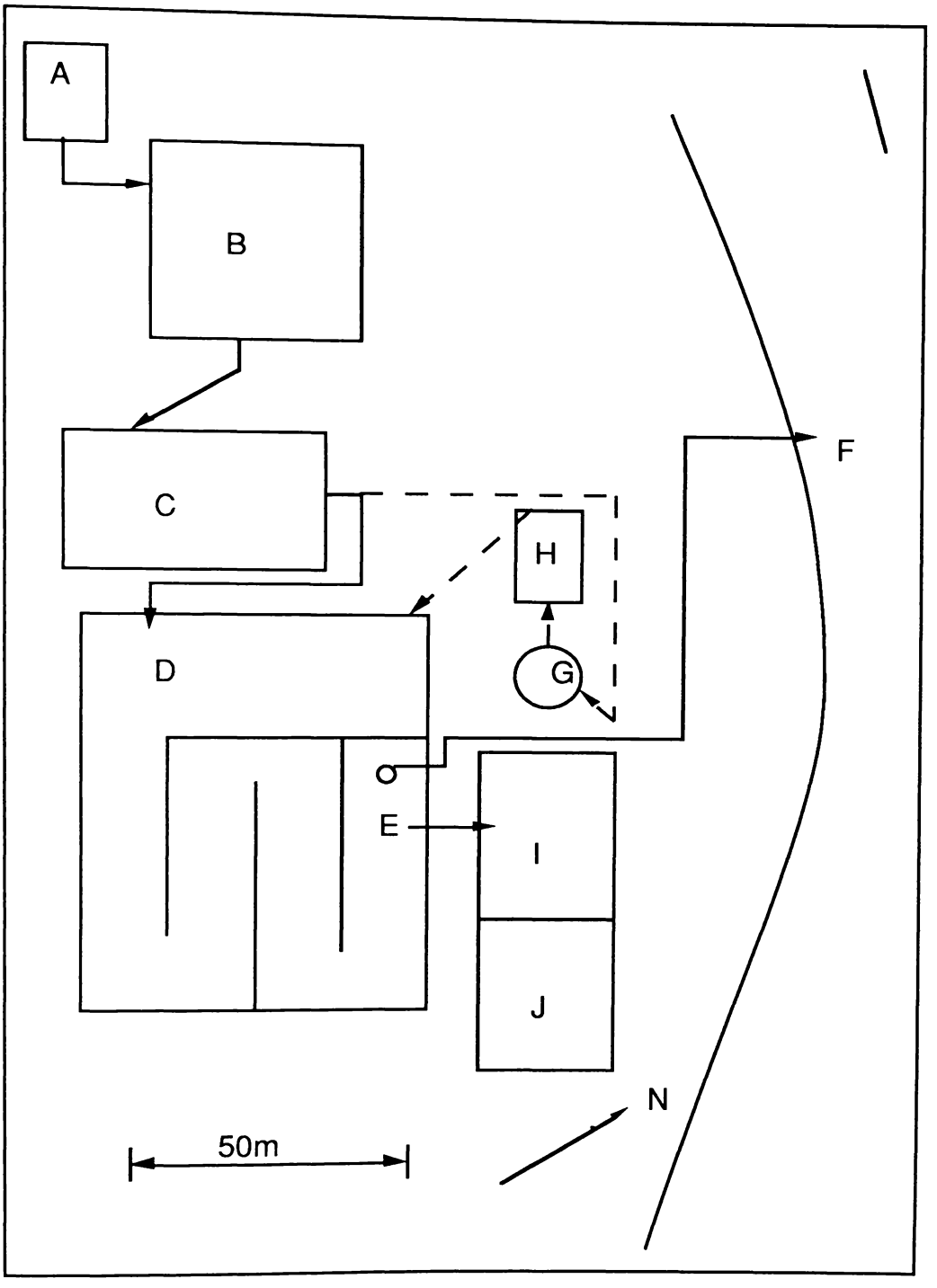

A
B
C
D
E
F
G
H
I
J
N
50m

Figure 3.2 : Diagrammatic representation of the layout of the sewage treatment system at the Affcco Meat Works, Horotiu. Effluent from the Meat works passes through primary treatment at (A) where fats are skimmed off the top and sludge is scraped from the bottom of the tank. The primary treated effluent is stored in (B) an anaerobic balance pond and is distributed to (C) an anaerobic pond at a constant rate. In these ponds anaerobic organisms digest the suspended and dissolved nutrients in the sewage. The digested sewage from (C) then passes into (D) where clarifiers aerate the sewage creating an aerobic environment and killing the anaerobes. The bulk of the effluent passes into (E) a facultative pond, with some sewage being diverted to (F) an aerated tank that distributes effluent to (G) experimental wetlands, before entering the facultative pond. In the facultative pond settlement of suspended particles and volatilisation of ammonia may occur. Effluent from the facultative pond is discharged into the Waikato River, with a small amount being sprayed onto an experimental forestry block.

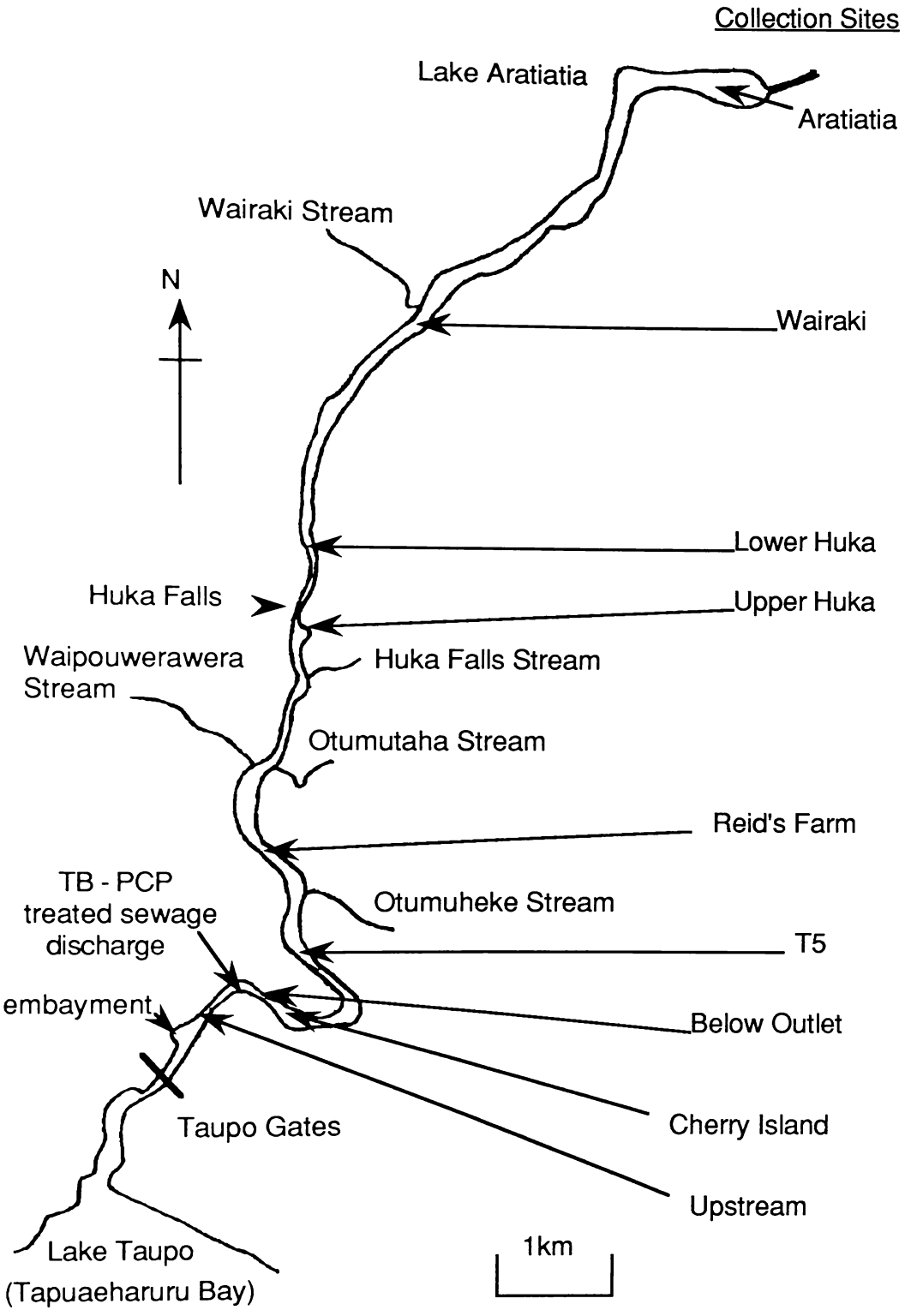


Figure 3.3 : Locality map of the Upper Waikato River study area, showing the inflow of major tributaries, the Taupo Borough Pollution Control Plant (TB - PCP) discharge into the river and the place names used in the text.

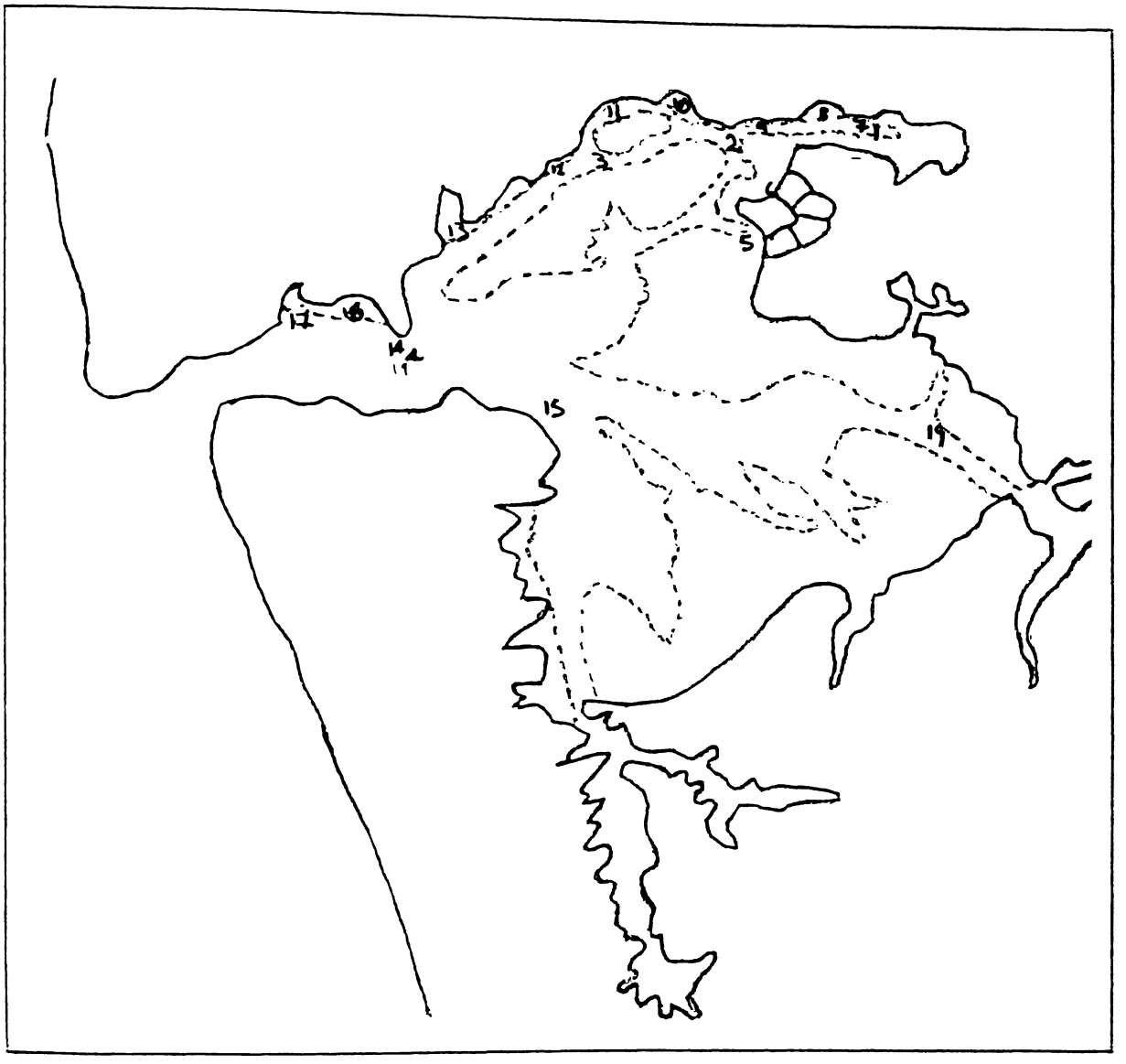


Figure 3.4 : Locality map of the Manukau Harbour showing the Manukau Sewage Purification Works and the sites where samples were collected from in the harbour. Sites 1 - 4 are from Vant's (1991) study of total nitrogen flows in the harbour, the other sites 5 - 19 are from this current study.

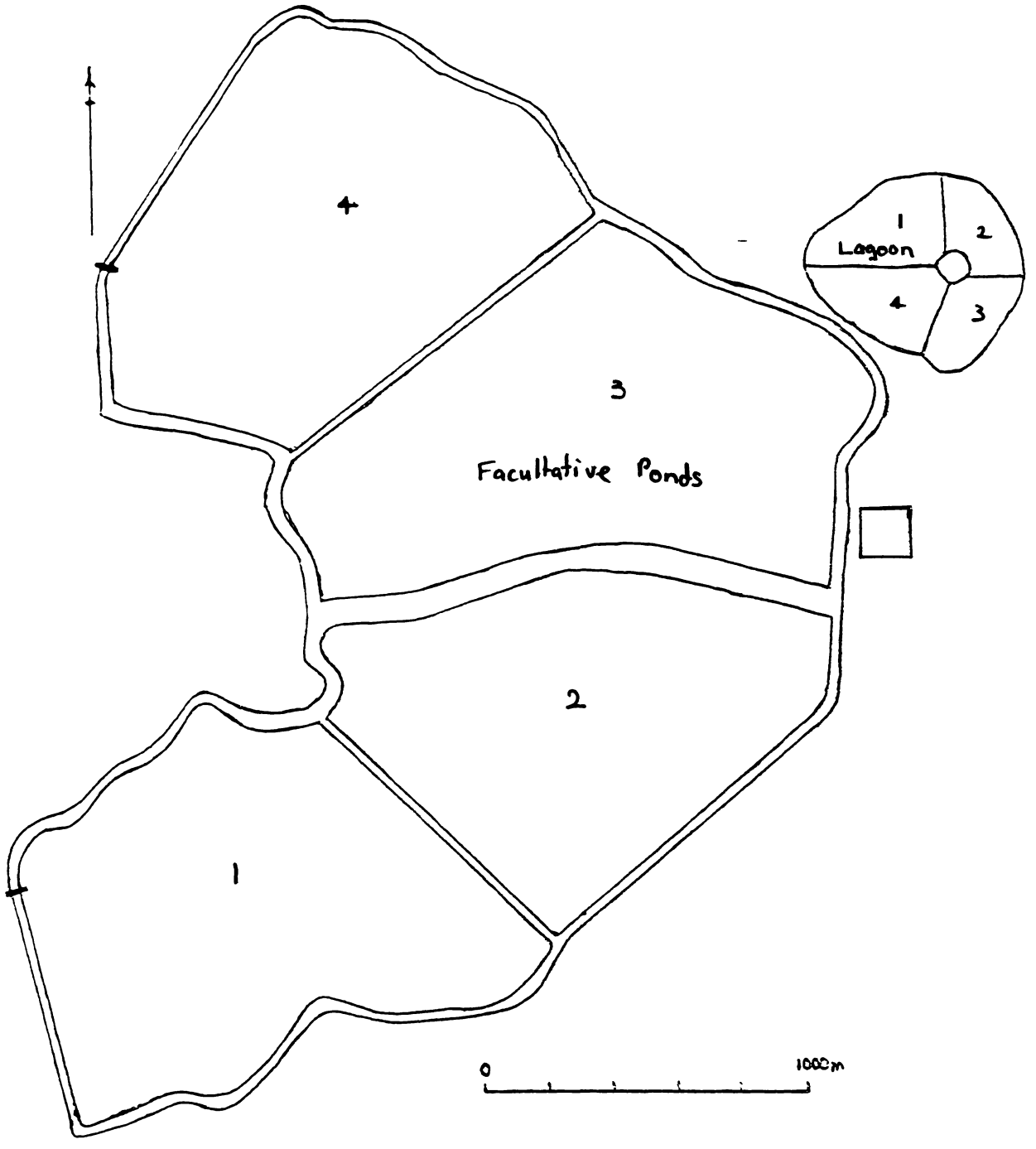


Figure 3.5 : Schematic diagram of the Manukau Purification Works showing the layout of the four facultative ponds and lagoons. Effluent outfalls from ponds one and four are indicated.

Chapter 4

^{15}N fractionation during sewage treatment

4.1 Preamble

Several studies have been reported in the literature of ^{15}N enrichment of NH_4 in treated sewage (Sweeney, 1978; Mariotti et al , 1984; Halley, 1990 and Miller, 1990), however little work has been carried out to investigate the cause of this phenomenon. The ^{15}N enrichment of sewage effluent may be an inherent property of raw sewage, or it may be the result of various processes in the treatment systems, or a combination of these. Therefore the purpose of this study is to carry out a preliminary investigation of the ^{15}N abundance of sewage NH_4 in various stages within a treatment plant and the isotope effect associated with nitrogen reactions.

The determination of sewage NH_4 ^{15}N abundance at specific points in the treatment plant enables an assessment of the likely cause of enrichment to be made. If (for example) the effluent discharged from a treatment plant was +10 $^{o}/_{oo}$ and the raw sewage was 0 $^{o}/_{oo}$, then we could conclude that the cause of the enrichment was due to processing. However if the effluent discharged from a treatment plant was +10 $^{o}/_{oo}$ and the raw sewage was also +10 $^{o}/_{oo}$, then the enrichment would appear to be an intrinsic property. Finally if discharged effluent was +10 $^{o}/_{oo}$ and the raw sewage was +4 $^{o}/_{oo}$, then it would seem that the enrichment was a combination of an inherent property and N process effect.

The isotope discrimination associated with various N processes can be determined both by indirect and direct methods. Examining the ^{15}N abundance and concentration of sewage NH_4 of both influent and effluent from a system can be used to determine the overall process discrimination of

^{15}N, from the mass balance of all the isotopes, before determining β. However to determine the isotope effect associated with a particular process, the products of the reaction must be collected and measured directly (eg. the products of uptake is the total plant nitrogen) and then β can be determined. Hence comparing the size of the isotope effect (β) for a particular process, with the β associated with the total N reaction in a sewage treatment system may determine the potential effect that a particular series of related reactions can make to effluent enrichment if it is the dominating effect.

4.2 During treatment

4.2.1 Discharged effluent

Ammonium is the predominant form of inorganic nitrogen in the treatment plant, and all the work described here concerns the isotopic analysis of ammonium N. Samples of liquid treated effluent from various plants were collected for analysis. The plants represent the main types of sewage treated in New Zealand.

Preparation of samples for analysis was by vacuum filtration on a Buchner funnel (GF/C filter) to produce an aqueous extract free from particulate materials. To test whether this process resulted in any significant alteration of ^{15}N levels a parallel test was run on wastewater which was settled in a separating funnel and decanted. The results (Table 4.1) show both preparation processes lead to the same ^{15}N levels, so the vacuum extraction technique was used as a standard method.

The results of ^{15}N abundances in the Horotiu, Te Aroha, Taupo, Manukau and Hamilton treated effluents are shown in table 4.2. All the effluents show values that are enriched compared with natural abundance.

Method	NH_4 -N (mg NH_4 l^{-1})	Atom % ^{15}N	$\delta^{15}N$
Vacuum Filtered	21.8 ± 1.0	.37368 ± .00009	+20.15 ± .24
Separation Funnel	22.7 ± 0.3	.37373 ± .00004	+20.26 ± .11

Table 4.1: The NH_4 -N concentration and ^{15}N abundance of a sewage sample that has been purified by the separation funnel and buchner apparatus techniques, before further processing using the Micromass method.

Treatment Plant	Atom % ^{15}N	$\delta^{15}N$
Horotiu	.37091 ± .00052	+12.6 ± 1.4
Te Aroha	.37370 ± .00025	+20.2 ± 0.7
Taupo (i)	.36927 ± .00015	+8.1 ± 0.4
(ii)	.36912 ± .00014	+7.7 ± 0.4
Manukau (i)	.37277 ± .00003	+17.7 ± 0.1
(ii)	.37368 ± .00009	+20.2 ± 0.2
Hamilton	.36892 ± .00052	+7.3 ± 1.4

Table 4.2 : Results of an investigation into the ^{15}N enrichment of NH_4 - N in final effluent collected from five treatment plants: Horotiu (2 / 10 / 90), Te Aroha (17 / 5 / 90), Taupo (i) (10 / 10 / 90), Taupo (ii) (4 / 2 / 91), Manukau (i) (15 / 1 / 91), Manukau (ii) (12 / 2 / 91) and Hamilton (15 / 1 / 91). The Hamilton, Taupo and Manukau plants treat predominantly domestic sewage, while the Horotiu and Te Aroha works treat Industrial effluent. from meat processing. The atom % ^{15}N and the ^{15}N in parts per mil ($^{o}/_{oo}$) is shown for each treatment station effluent.

4.2.2 Within plant

The isotope effect associated with the overall treatment plant processing can be broken down to the discrimination associated with each stage in the procedure.

Wastewater samples were collected from various stages within the Horotiu (fig. 3.2) and Manukau (fig. 3.4) treatment plants. At Horotiu wastewater from the primary, anaerobic and facultative ponds were collected. In the Manukau plant liquid treated sewage samples were collected from facultative ponds: 2 (Inlet and outlet), 4 (outlet) and the sludge ponds one and four. The wastewater samples were processed by the standard procedures.

The result of ^{15}N abundance within the Horotiu and Manukau treatment plants are shown in tables 4.3a and 4.3b. The sewage effluent entering the Horotiu sewage treatment plant contains a moderate level of ammonium (12.7 mg N l^{-1}), which is enriched in ^{15}N (+4.1 $^{o}/_{oo}$). However as the effluent passes through the treatment plant the NH_4 - N concentration increases to 106 mg N l^{-1} (facultative pond influent), which is slightly reduced (to 95 mg N l^{-1}) before the sewage is discharged into the Waikato River. Enrichment of ^{15}N is most significant in the facultative pond effluent (+13.3 $^{o}/_{oo}$), although the biggest change occurs between the primary and anaerobic pond (+4.1 to +10.7 $^{o}/_{oo}$).

In the Manukau plant (Table 4.3b) wastewater mixed in with the sludge separated during primary treatment contains a high concentration of NH_4 - N (103.7 mg N l^{-1}) and is highly enriched in ^{15}N (+34.8 $^{o}/_{oo}$). Sewage that is circulated through the facultative ponds which have a lower concentration of NH_4 - N (20 - 25 mg N l^{-1}), and are also enriched in ^{15}N (+15 to +20 $^{o}/_{oo}$).

A

Site	mg NH_4 l^{-1}	Atom % ^{15}N	$\delta^{15}N$
Primary Treatment Tank	12.7 ± .08	.36781 ± .00033	+4.11 ± .91
Anaerobic Pond (Effluent)	106 ± 2	.37023 ± .00003	+10.74 ± .07
Facultative Pond (Effluent)	95 ± 2	.37117 ± .00003	+13.30 ± .09

B

System	NH_4 - N (mg N l^{-1})	Atom % ^{15}N	$\delta^{15}N$
Lagoon 4 (i)	103.7 ± 5.8	.37903 ± .00004	+34.8 ± 0.1
Pond 2 (I) (i)	25.2 ± 2.4	.37212 ± .00009	+15.9 ± 0.5
Pond 2(O) (i)	20.4 ± 0.3	.37301 ± .00018	+18.3 ± 0.5
(ii)	22.1 ± 1.4	.37312 ± .00003	+18.6 ± 0.1
Pond 4 (O) (i)	21.6 ± 0.6	.37277 ± .00003	+17.7 ± 0.8
(ii)	21.3 ± 0.9	.37368 ± .00009	+20.2 ± 0.2

Table 4.3a : The concentration and ^{15}N abundance of NH_4 - N in sewage, collected ((i) 15 / 1 / 91 and (ii) 12 /2 / 91) from various segments in the Horotiu treatment plant (fig. 3.2) .

Table 4.3b : The concentration and ^{15}N abundance of sewage NH_4 - N in various segments in the Manukau Purification Works (fig. 3.4). Effluent samples were collected on two occasions ((i) 15/1/91 (ii) 12/2/91).

4.3 Modelling Process Fractionation

4.3.1 Preamble

In considering the isotopic effects of various processes on N enrichment in treated sewage, several factors that affect the size of the ammonium pool should be taken into account. The NH_4 existing in the raw sewage may be added to, by ammonium produced from the breakdown of organic - N compounds during bacterial degradation. Balancing this (at least partially is the removal from the system of NH_4 (eg. NH_3 volatilisation) or the conversion to other N forms (eg NO_3 and bacterial immobilisation). However it is impossible to separate these processes so that their influence on overall enrichment may be determined. The size of the fractionation associated with the various individual N processes involved, is known from a range of N cycle studies (see Chapter 1), however their magnitude of expression in a sewage treatment plant has not been previously studied. Thus the purpose of this study is to carry out a preliminary investigation of the apparent isotope effect of the average process and the actual fractionation associated with specific processes in the facultative segment of the sewage treatment system.

In the facultative pond the most significant fractionation process would be expected to occur during nitrification, due to the high levels of NH_4 (Table 4.3) and the low β associated with this reaction (Section 1.4.2.2). However we have no way of measuring the effect of nitrification, because the concentration of nitrate is low and the products produced can not be isolated without being affected by other processes. Hence the fractionation associated with two other processes known to occur in the facultative pond (algal NH_4 - assimilation and NH_3 - volatilisation) have been determined. Thus we may be able to obtain an indication of the significance of the overall process fractionation within the pond and also the discrimination for several specific processes.

4.3.2 Apparent N process effect

It is possible to determine β (eq. 1.5) the fractionation due to N processes, when the isotopic ratio of the reactant nitrogen and products are known. Thus uptake of ammonium into algal cells discriminates against ^{15}N, the algal cells have a lower ^{15}N enrichment eg. for the facultative ponds: NH_4 in the ponds is 0.36924 and N in the algae is 0.36622, therefore β is .9918 (Table 4.4a). However within the total processes of a treatment plant we may know for example the concentration and ^{15}N levels of the influent and effluent NH_4 of any segment of the system, but we do not know the ^{15}N abundance or the concentration of the products of the many fractionation processes occuring within that segment.

The concentration of influent and effluent NH_4 to a segment of a treatment system is known and therefore the products can be calculated (ie. [influent NH_4] = [effluent NH_4] + [product NH_4] therefore [product NH_4] = [influent NH_4] - [effluent NH_4]). The isotope composition of NH_4 - N for the influent and effluent is also known, therefore multiplying the ^{15}N abundances by the respective ammonium concentrations the product ratio is the only unknown in the mass balance equation (eq. 4.1). The formula (eq. 4.1) can be rearranged so that the ^{15}N abundance of the products can be determined (eq. 4.2).

$$a(A) = b(B) + c(C) \qquad \text{(eq. 4.1)}$$

The quantity of initial substrate (ie. influent NH_4 - N concentration) multiplied by it's ^{15}N abundance (in Atom %) equals the quantity of final substrate, multiplied by it's ^{15}N abundance, plus the quantity of final substrate, multiplied by it's ^{15}N abundance.

Key:

a : Influent NH_4 concentration

b : Effluent NH_4 concentration

c : Product NH_4 concentration

A : ^{15}N abundance of the Influent NH_4 in Atom %

B : ^{15}N abundance of the Effluent NH_4 in Atom %

C : ^{15}N abundance of the product NH_4 in Atom %

This can be arranged to express the unknown (ie product isotope composition) as:

$$(C) = \frac{a(A) - b(B)}{c} \qquad \text{(eq. 4.2)}$$

Example : If the influent and effluent from a sewage treatment system have the NH_4 - N concentrations of 50 ± 1 (standard units) and 45 ± 1 (relative to the influent concentration), and ^{15}N abundances of .36800 ± .00005 and .36900 ± .00005 (Atom %), the concentration of the product is the difference between the influent and effluent. The isotope effect can be calculated as follows:

$$(C) = \frac{18.4 \pm 1.00005 - 16.605 \pm 1.00005}{5 \pm 2} = .3590 \pm .0001$$

Thus the the ^{15}N abundance of the average products produced in this segment of a treatment system is .3590 ± .0001 Atom %.

and:

$$\beta = ((C)/(A) + (C)/(B))/2$$

$$= .97422 \pm .00005$$

Using the formula (Eq. 4.2) the ^{15}N apparent abundance of the average products can be calculated fro the facultative ponds at Horotiu and Manukau.

The concentration and ^{15}N abundance of the influent NH_4 into the Horotiu facultative pond, was 106 ± 2 mg N l^{-1} with .37023 ± .00003 Atom % ^{15}N. The concentration and ^{15}N abundance of the effluent NH_4 from the facultative pond, was 95 ± 2 mg N l^{-1} with .37117 ± .00003 Atom % ^{15}N. Hence the amount of product - N produced by the combination of various processes is 11 ± 4 mg N l^{-1} . Therefore the ^{15}N abundance of the average product from various N - processes in the facultative pond is .36211 Atom % ^{15}N with a standard error of ± .00006 (ie - 11.4 ± 0.2 $^{o}/_{oo}$) thus the average isotope effect associated with N - processes over the entire system is β =.9768 ± .00003.

The concentration and ^{15}N abundance of the influent NH_4 into the Manukau facultative pond (number two) was 25.2 ± 2.4 mg N l^{-1} with .37212 ± .00009 Atom % ^{15}N. The concentration and ^{15}N abundance of the effluent NH_4 from the facultative pond, was 20.4 ± 0.3 mg N l^{-1} with .37301 ± .00018 Atom % ^{15}N. Hence the amount of product - N produced by the combination of various processes is 4.8 ± 2.7. Therefore the ^{15}N abundance of the average product from various N - processes in the facultative pond is .36834 Atom % ^{15}N with a standard error of ± .00027 (ie + 5.6 ± 0.7 $^{o}/_{oo}$) thus the average isotope effect associated with N - processes over the entire facultative pond is β =.9887 ± .0001.

4.3.3 *Insitu* Algae NH_4 assimilation

A variety of biological reactions have been shown to discriminate against ^{15}N, thus in a preliminary investigation to estimate the β associated with these processes in a sewage treatment plant *insitu* NH_4 assimilation by algae was studied. Ammonium is present in sewage at very high concentrations (20 - 40 $mgNl^{-1}$), and therefore the K_m of *insitu* algae assimilation is saturated by several orders of magnitude. The relative proportion of NH_4 removed is small compared with the ammonium available, resulting in the maximum isotope effect occuring (Section 1.3.2). The β for NH_4 assimilation by *insitu* algae in a variety of treatment plants has been assessed to determine the potential that this process has in contributing to the isotopic enrichment of NH_4 in a sewage treatment plant .

Sewage samples were collected from various systems within the Manukau, Taupo and Hamilton treatment plants. In the Manukau treatment plant effluent from the facultative ponds two and four (both samples taken from the outlets) and an old sludge lagoon (number four) were collected. Samples from the Hamilton treatment plant were obtained from unchlorinated and chlorinated systems. Taupo effluent was collected from a final clarity pond in the treatment system. All sewage samples were preserved and processed by the prescribed method for the Micromass ^{15}N analysis procedure.

The results from processing samples by the micromass ^{15}N analysis method (Table 4.4), show that the nitrogen content of *insitu* algae in various treatment ponds from the Taupo, Hamilton and Manukau treatment plants, ranged from: 7.7 to 8.2 %N (Taupo), 6.1 to 6.3 %N (Hamilton) and 4.08 to 4.85 %N (Manukau). The variation in β for the N isotope ratio between *insitu* algae and sewage effluent in the treatment plants was: .9898 to .9911 (Taupo), .9918 to .9932 (Hamilton) and .9781 to .9874 (Manukau).

A

Segment	N - content	Atom % ^{15}N	$\delta^{15}N$	β
Chlorinated	28 ± 2 mgl^{-1}	.36924±.00009	+8.0 ± 0.2	-
Algae	6.3 %	.36622	- 0.22	.9918 ± .0003
Unchlorinated	38 ± 5 mgl^{-1}	.36871±.00015	+6.6 ± 0.4	-
Algae	6.1 %	.36672±.00002	+1.15 ± .05	.9932 ± .0003

B

Segment	N - content	Atom % ^{15}N	$\delta^{15}N$	β
Clarity (i)	60.5±1.4 mgl^{-1}	.36927±.00051	+8.1 ± 1.4	-
Algae A	-	.36598±.00009	- 0.9 ± 0.2	.9911 ± .0016
Algae B	-	.36549±.00011	- 2.2 ± 0.3	.9898 ± .0016
Clarity (ii)	33.4±0.7 mgl^{-1}	.36912±.00042	+7.7 ± 1.1	-
Algae A	8.2 %	.36574±.00018	-1.5 ± 0.5	.9909 ± .0014
Algae B	7.7 %	.36538±.00014	-2.5 ± 0.4	.9899 ± .0013

C

Segment		N - content	Atom % ^{15}N	$\delta^{15}N$	β
Lagoon 4	(i)	103.7±5.8mgl^{-1}	.37903± 00004	+34.8 ± 0.1	-
Algae	(i)	-	.37144±.00010	+14.0 ± 0.3	.9800 ± .0003
Pond 2 (I)	(i)	25.2 ± 2.4mgl^{-1}	.37212±.00009	+15.9 ± 0.5	-
Algae	(i)	-	.36549±.00005	-2.2 ± 0.1	.9821 ± .0002
Pond 2 (O)	(i)	20.4 ± 0.3mgl^{-1}	.37301±.00018	+18.3 ± 0.5	-
	(ii)	22.1 ± 1.4mgl^{-1}	.37312±.00003	+18.6 ± 0.1	-
Algae	(i)	-	.36482±.00003	-4.0 ± 0.4	.9781 ± .0005
	(ii)	4.85 ± .05 %	.36842±.00013	+5.8 ± 0.4	.9874 ± .0004
Pond 4 (O)	(i)	21.6± 0.6mgl^{-1}	.37277± 00003	+17.7 ± 0.8	-
	(ii)	21.3 ± 0.9 mgl^{-1}	.37368±.00009	+20.2 ± 0.2	-
Algae	(i)	-	.36555±.00005	-2.0 ± 0.1	.9806 ± .0002
	(ii)	4.08 ± .04 %	.36741±.00022	+3.0 ± 0.6	.9829 ± .0007

Table 4.4a: ^{15}N enrichment of effluent and *insitu* algae collected (15 / 1 / 91) from the Hamilton treatment plant's unchlorinated and chlorinated sewage ponds. N - content, Atom % ^{15}N and $\delta^{15}N$ values for sewage and *insitu* algae are shown, and the isotope effect β.

Table 4.4b : ^{15}N enrichment of effluent and *insitu* algae collected ((i) 10 / 10 / 90 and (ii) 4 /2 / 92) from the Clarity pond at the Taupo Borough Pollution Control Plant. N - content, Atom % ^{15}N and $\delta^{15}N$ values for sewage and *insitu* algae samples A and B, (Appendix C1) are shown, and the isotope effect β.

Table 4.4c : ^{15}N enrichment of effluent and *insitu* algae collected ((i) 15 / 1 / 91 and (ii) 12 /2 / 91) from various ponds in the Manukau Purification Works. N - content, Atom % ^{15}N and $\delta^{15}N$ values for sewage and *insitu* algae samples (Appendix C1) are shown, and the isotope effect β.

4.3.4 Ammonia Volatilisation

Ammonia volatilisation is a process that results in significant discrimination under certain conditions, these are similar to what is found in a treatment plant facultative pond (ie high ammonium concentration and pH) (see Chapter 1). Hence the fractionation during ammonia volatilisation from a pond would be expected to be high. In order to test this hypothesis air above the facultative pond at Horotiu (fig 3.2) was collected and analysised.

To sample volatilised NH_3, air was drawn through a tube floating on the facultative pond and into a NH_3 trap containing glass balls and 2% phosphoric acid. An upside down two litre icecream container supported on a polystyrene base served as the support structure for the tube on the pond. Air collected via a plastic tube attached to the top of the icecream container, passed through a rubber bung to within five mm of the bottom of a measuring cylinder (ie. NH_3 trap). At the end of the inlet tube a porous stone dispersed the air through the phosphoric acid and glass balls, where ammonia was trapped before the air minus NH_3 was released into the atmosphere. The system was set to run at a flow rate of 7.3 litres / min. over four days. In laboratory tests, two measuring cylinders containing phosphoric acid and glass balls were connected in series to test the effectiveness of the trapping method. It was found that all the NH_3 - N in an air sample was collected in the first trap. Air samples from over the Horotiu facultative pond (fig 3.2) and background values over grass (10 m from the pond) were sampled at the same time. Air and effluent temperatures, and the waste water pH were measured at both the initiation and completion of a sample period. At the end of a sampling period, the traps were removed. The acid was eluted with water, and the NH_3 - N concentration was determined using standard procedures.

A second method for trapping atmospheric ammonia was trialed, using 500 mls of phosphoric acid spread over a tray. Three trays were prepared and placed on a platform surrounding the Horotiu treatment plant's facultative

pond's outlet (fig. 3.2), and left for four days before collection and analysis of trapped ammonia.

The concentration of volatilised NH_3 collected by the measuring cylinder traps ranged from 103 to 483 $\mu g / m^3$ for eight samples with a mean of 284 $\mu g / m^3$ and S. D. of 157 $\mu g / m^3$. The ^{15}N abundance of the ammonia (compared with natural abundance) varied from -36.7 to -6.4 $^o/_{oo}$, with a mean of -18.7 $^o/_{oo}$ and S. D. of 10.1 $^o/_{oo}$. The relationship between the concentration and the ^{15}N abundance of the volatilised ammonia is shown in fig. 4.1. The β for the ratio of atmospheric NH_3 to aqueous NH_3 - N varied from .9506 to .9806, with a mean of .9685 and S. D. of .010. The pH of the wastewater during the sampling period varied from 7.6 to 8.2 with a mean of 7.8 and S. D. of 0.2, while the temperature differential (atmospheric - effluent) over the same time ranged from -7 ^oC to 4 ^oC with a mean of -1.9 ^oC and S. D. of 3.2 ^oC. The concentration of background NH_3 (fig. 4.2) collected over grass, 10 m from the facultative pond at the Horotiu sewage treatment plant ranged from 11.9 $\mu g / m^3$ to 49 $\mu g / m^3$ for eight replicated samples, with a mean of 19.8 $\mu g / m^3$ and S. D. of 8 $\mu g / m^3$. The ^{15}N abundance of the background ammonia varied from -7.6 to +3.5 $^o/_{oo}$, with a mean of -4.1 $^o/_{oo}$ and S. D. of 4.6 $^o/_{oo}$.

The concentration of NH_3 in the three acid trays ranged from 0.15 to 2.919 mg, with a mean of 1.94 mg and S. D. of 1.55 mg. The ^{15}N abundance of the ammonia (compared with natural abundance) varied from -50.3 to -47.1 $^o/_{oo}$, with a mean of -49.2 $^o/_{oo}$ and S. D. of 0.7 $^o/_{oo}$. The β for the ratio of atmospheric NH_3 to aqueous NH_3 - N varied from .9371 to .9404, with a mean of .9384 and S. D. of .0018.

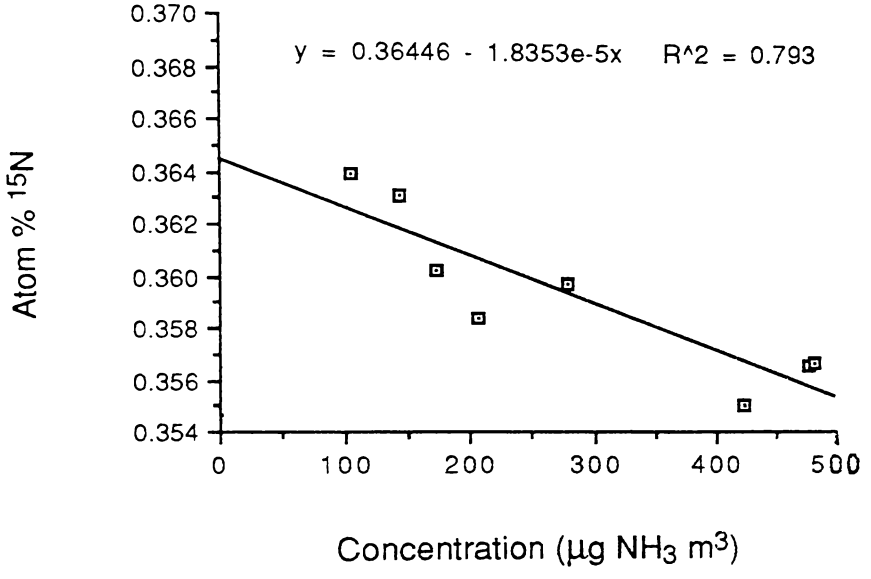


Figure 4.1 : Isotopic abundance of NH_3 volatilised from the Horotiu facultative pond (fig. 3.2) with respect to atmospheric concentration of ammonia.

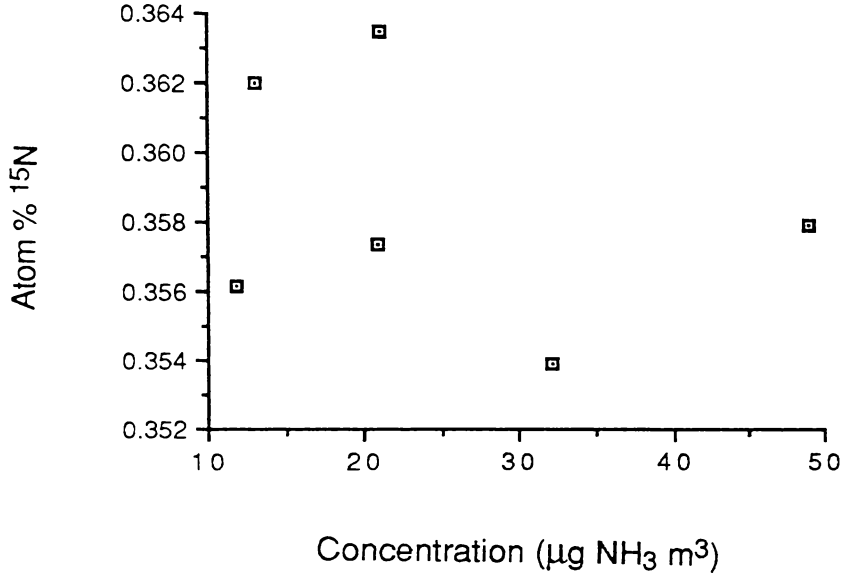


Figure 4.2 : Isotopic abundance of background NH_3 collected 10 m from the Horotiu facultative pond (fig. 3.2) with respect to atmospheric concentrations of ammonia.

Chapter 5

Tracing the impacts of wastewater N in a receiving body

5.1 Preamble

Numerous studies have documented the deterioation of water quality in lakes, rivers and esturaries receiving sewage inputs, which results in high levels of macrophyte and algal growth (eg. Bothwell, 1985; Soulsby et al, 1985; Richardson and Schwelger, 1986; Makinen and Aulio, 1986; and Miller et al, 1988 and Jupp and Spence, 1977). Several factors may be linked to these changes, eg. temperature, light and nutrients. Of these, nutrient levels (especially N and P) are often the dominating factor associated with increased growth rates. This chapter presents the isotopic values of plant N in the Upper Waikato River (UWR) and the Northern Manukau Harbour (NMH).

The UWR has been well documented for it's flora composition and distribution before (Coffey, 1979) and after (Miller, 1990) it's use as a receiving body for treated effluent. The diversity of flora downstream from the effluent discharge is now significantly lower that in upstream (uncontaminated) sites. Downstream sites that would previously have been occupied by small native species now contain members of the Hydrocharitaceae (*Elodea canadensis, Lagarosiphon major , Egeria densa* and *Certophyllum demersum*) (Howard - Williams and Davies, 1988). These tall growing species often have the perphytic algae *Enteromorpha nana* attached to them (this relationship does not occur upstream) which is thought to be a result of the nutrient status of the water (Miller, 1990).

In the Manukau Harbour effluent from the Manukau treatment plant flows into the north harbour, this region is characterised by the presence of the large

red alga *Gracillaria secundata pseudoflagellifera* which is absent from the rest of the harbour (Johnson, personal communication).

5.2 Upper Waikato River

5.2.1 Isotope Abundance of Aquatic Plants

A preliminary attempt at applying ^{15}N methodology in the Upper Waikato River showed that L. major was enriched in ^{15}N downstream compared with upstream plants. An investigation (August, 1990) of the natural abundance levels found in a wide range of water plants growing on a variety of substrates and sites was carried out to assess whether this phenomenon was typical of all plant species in the river, and to determine whether there was any variation between species and sites.

Samples of various plant species were collected from an upstream site and several downstream locations (fig 3.3). The samples were processed using standard procedures and the results are shown in Tables 5.1 - 5.5.

The N isotopic ratio for Upstream plants (Table 5.1) shows that there is considerable variation between species (up to 6.69 $^{o}/_{oo}$). The range of values expressed have a mean of + 0.2 $^{o}/_{oo}$ (similar to atmospheric natural abundance) with a spread from -3.33 to 3.36 $^{o}/_{oo}$. Vegetation categories show almost the same amount of variation as the overall community (eg tall macrophytes 5.56 $^{o}/_{oo}$).

Table 5.2 lists the isotopic values for downstream plants. All the plants collected were enriched in ^{15}N (except *Potamogeton cheesmanni*) with respect to atmospheric natural abundance (ie +1.20 to +3.78 $^{o}/_{oo}$). Both L. major and E. nana have a higher isotopic ratio downstream than upstream.

Plant Species Collected	kN%	Atom % ^{15}N	$\delta^{15}N$ (‰)
Glossostigma diantrum	3.05	.36697	+1.83
Litaeopsis ruthiana	2.66	.36508	-3.33
Myriophyllum triphyllum	2.35	.36671	+1.12
Fissidens rigidulus	0.83	.36607	-0.63
Tridontium tasmanicum	0.95	.36577	-1.45
Chara fibrosa	2.74	.36630	+0.00
Lagarosiphon major	2.41	.36580	-1.37
Myriophyllum votschi	2.66	.36699	+1.88
Potamogeton cheesmanni	3.78	.36542	-2.40
Ranunculus trichophyllus	2.97	.36753	+3.36
Enteromorpha nana	1.03	.36727	+2.65
Nostoc spp.	3.35	.36651	+0.57
Oedogonium spp.	0.63	.36665	+0.96
Upstream average	2.26 ± .57	.36637±.00040	+0.2 ± 1.1

Table 5.1: Nitrogen content and ^{15}N abundances for plants upstream of the Taupo Borough Pollution Control Plant effluent discharge into the Waikato River (fig. 3.3).

Site : Species Collected	kN%	Atom % ^{15}N	$\delta^{15}N$ (‰)
A:Lagarosiphon major	2.62	.36729	+4.07
A:Enteromorpha nana	3.27	.36855	+5.41
A:Vaucheria spp.	1.08	.36674	+1.20
B:Enteromorpha nana	3.65	.36807	+4.83
B:Cladofera glomerata	2.51	.36733	+2.81
C:Enteromorpha nana	3.32	.36885	+6.96
D:Enteromorpha nana	3.01	.36868	+6.50
E:Lagarosiphon major	3.53	.36728	+2.68
E:Potamogeton cheesmanni	4.10	.36532	-2.68
E:Enteromorpha nana	1.52	.36871	+6.58
F:Nitella spp.	2.54	.36643	+0.35
F:Lagarosiphon major	2.63	.36701	+1.94
F:Enteromorpha nana	2.12	.36699	+1.88
F:Gomphonema spp.	1.67	.36686	+1.53
Downstream average	2.68 ± .46	.36742±.00052	+3.2 ± 1.4

Table 5.2 : Nitrogen content and ^{15}N abundances for plants downstream of the Taupo Borough Pollution Control Plant effluent discharge into the Waikato River. Sample sites were: (A) Below Outlet, (B) T5-site, (C) Reid's Farm, (D) Upper Huka Falls, (E) Lower Huka Falls and (F) Wairaki (fig. 3.3).

5.2.2 Plant - N as an Indicator of Source

The study by Miller showed that *Lagarosiphon major* and *Enteromorpha nana* were present both upstream and downstream of the Taupo effluent discharge (Miller, 1990). L. major represents the closest example to a true aquatic macrophyte, while E. nana is a periphytic algae, both of these plants are often sited in effluent studies.

Samples of E. nana and L. major were collected (August, 1990) from various sample sites in the Upper Waikato River and processed using the Micromass method (Section 2.4.2). The sample sites were Upstream, Below Outlet, T5 site, Reid's Farm, Upper Huka Falls, Lower Huka Falls and Wairaki (see fig 3.3). At each site, a single sample from each species (if present) was collected and later processed.

^{15}N analysis of E. nana (Table 5.3a) showed that plants downstream of the effluent discharge were +2 - 4 $^{o}/_{oo}$ more enriched than upstream. Upstream E. nana is found only in an embayment that used to be the former river channel, this is affected by runoff and therefore the isotopic value may be different to that in the main channel. Downstream, the smallest enrichment (cf. upstream) was observed in the two sites closest to the effluent outlet, more distant sites showed the highest isotope ratios, although a clear trend of increasing enrichment with distance downstream was not evident.

In L. major (Table 5.3b), enrichment below the effluent outlet varied from +3 - 5.5 $^{o}/_{oo}$, with an apparent decrease in enrichment with distance downstream. This trend is not what would have been expected, isotope theory indicates that enrichment should increase in the products (ie. plant - N) that is produced later if an isotopic effect is occuring (see Chapter 1), if there is no discrimination the substrate will remain at a constant ^{15}N abundance and products produced should have the same values.

A second study was carried out on L. major and it's close relative *Elodea canadensis* (both are members of the Hydrocharitaceae family). Both species have similar growth forms and therefore would be expected to be similar isotopically. Samples were collected (August, 1991) in triplicate at the Upstream, Below Outlet, Cherry Island, Reid's Farm, Upper Huka Falls, Lower Huka Falls, Wairaki and Aratiatia sites (see fig. 3.3) and processed using the tracer mass method (Section 2.4.3).

Results (fig. 5.1) have been graphed as enrichment and nitrogen content versus distance from the Taupo Gates downstream. For L. major enrichment increases from 1.2$^{o}/_{oo}$ upstream to 3.5 $^{o}/_{oo}$ immediate below the outlet, and continues to increase downstream, to a maximum of +8.1 $^{o}/_{oo}$ (Wairaki site) before decreasing to +4.0 $^{o}/_{oo}$ at Aratiatia. Plant nitrogen content, remains relatively constant for all the sites (Upstream and Downstream).

E. canadensis (fig. 5.1) shows a similar trend to L.major with an initial increase in enrichment downstream. Values obtained for the Upper and Lower Huka falls sites are similar to L. major, giving us increased confidence in their magnitude. Plant nitrogen content is higher than L. major, but shows no significant variation between sites.

A

Site:	kN%	Atom % ^{15}N	$\delta^{15}N$
Upstream	1.03 ± .02	.36727±.00020	+2.65 ± .55
Below Outlet	1.42 ± .48	.36836±.00022	+5.62 ± .60
T5 site	3.68 ± .06	.36807±.00014	+4.83 ± .38
Reid's Farm	3.29 ±.06	.36885±.00012	+6.96 ± .33
Upper Huka Falls	3.02 ± .03	.36872±.00006	+6.61 ± .16
Lower Huka Falls	1.64 ± .37	.36859±.00018	+6.25 ± .49
Wairaki	2.12 + .25	.36899+.00011	+7.34 ± .30

B

Site:	kN%	Atom % ^{15}N	$\delta^{15}N$
Upstream	2.58 ± .14	.36580±.00018	-1.37 ± .49
Below Outlet	3.10 ± .60	.36786±.00014	+4.26 ± .38
Lower Huka Falls	3.73 ± .60	.36731±.00014	+2.76 ± .38
Wairaki	2.92 ± .22	.36697±.00017	+1.83 ± .46

Table 5.3 (a and b) : ^{15}N abundance and tissue Nitrogen content of whole plant, *Enteromorpha nana* on Lagarosiphon (a) and *Lagarosiphon major* (b) growing at various sites in the Upper Waikato River. The ^{15}N abundance is shown as Atom % and as a comparison with atmospheric dinitrogen (δ). Fig. 3.3 shows the collection site locations in the Upper Waikato River.

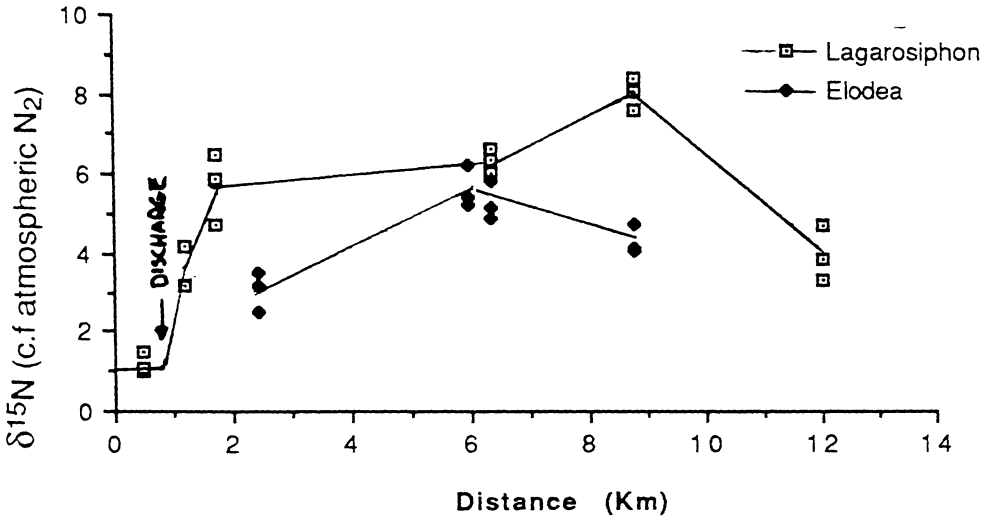


Figure 5.1 : *Lagarosiphon major* and *Elodea canadensis*, ^{15}N enrichment in the Upper Waikato River with respect to distance downstream from the Taupo Gates. The point where treated effluent from the Taupo Borough Pollution Control Plant is discharged is and sample sites are indicated (fig. 3.3).

5.3 Northern Manukau Harbour

5.3.1 Gracillaria

G. secundata was gathered from a variety of sites in the northern harbour on two occasions (12 / 2 / 91 and 9 / 8 / 91). Samples were collected from near the major (outlet 1) and minor (outlet 2) effluent discharging points from the Manukau treatment plant, Onehunga, Blockhouse Bay, Green Bay and Little Muddy Creek. The location of the sites in the northern harbour is shown in fig. 3.4. Samples collected on the first outing were processed by the micromass method (Section 2.4.2) while the others (collected 9 / 8 / 91) were prepared by the Europa Tracermass method (Section 2.4.3).

The relationship between the nitrogen isotopic ratio and content for G. secundata in the harbour, with respect to distance from the treatment plant is shown in fig. 5.2. There appears to be a correlation between distance from the treatment plant and the percentage N content, with an inverse relationship occuring between percent N and it's isotopic value. Near the treatment plant the N content is high and the isotope values are low, as the distance for the plant increases the N content decreases and the isotope ratio increases

5.3.1 Harbour Sediments

Harbour sediments were collected from 13 sites in the Manukau harbour on two occasions (12 / 2 / 91 and 9 / 8 / 91). Samples were gathered from near Outlet 1 and Outlet 2, Hillsborough, Wesley Bay, Onehunga, Faulkner Bay, Blockhouse Bay, Green Bay, French Bay, Little Muddy Creek, Armour Reserve and Huia (sites are shown in fig 3.4). Nitrogen in the samples was first extracted and concentrated using the Kjeldahl method (Section 2.4.2.2) before being processed using the tracermass analysis method (Section 2.4.3).

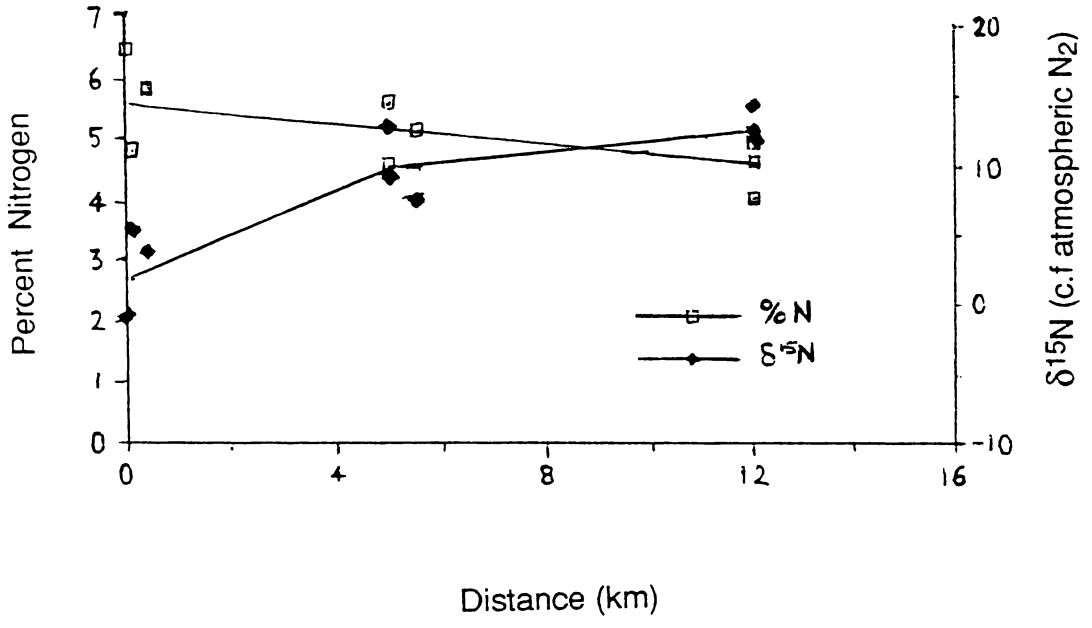


Figure 5.2 : *Gracillaria secundata*, ^{15}N abundance and percentage N - content in the Northern Manukau Harbour with respect to the distance from the Manukau Purification Works via the circulation channels (fig. 3.4).

Figure 5.3 shows the relationship between N content and isotopic ratio versus the distance from the Manukau treatment plant in harbour sediment. A similar trend as observed in G. secundata is found (although the percentage N content is lower), sediment near the treatment plant (high N content) has a low ^{15}N abundance with the N content decreasing away from the station and it becoming more enriched.

5.3.1 Suspended Organic Matter

Water samples were collected from five sites in the northern harbour and one site within the Manukau treatment station. The sampling sites were: the facultative pond no. four (within the treatment station), Onehunga, French bay, Weymouth, Waiuku Channel and Puponga Point (within the harbour). All the sampling locations are shown in the map of the northern harbour (fig. 3.4). Samples were centrifuged to extract the suspended organic matter (SOM) which was then analysed by the tracermass method for N isotope determination (Section 2.4.3).

Table 5.4 shows the N content and ^{15}N values for SOM in the Manukau Harbour. The highest N content was found in the facultative pond (7.58%) which is much higher than in the harbour (c. 0.3%). N content in the harbour is remarkable consistent for all the samples collected (ie. 0.26 - 0.31%). The isotopic ratio in the treatment pond is depleted (-6.6 $^{o}/_{oo}$), however in the harbour, all samples are enriched. Samples in or near the northern harbour are the lowest (ie. Onehunga +6.9, French Bay +5.6 and Waiuku +6.5$^{o}/_{oo}$) with higher values observed at Weymouth (+12.0 $^{o}/_{oo}$) and the harbour entrance channel (Puponga Point +10.6 $^{o}/_{oo}$).

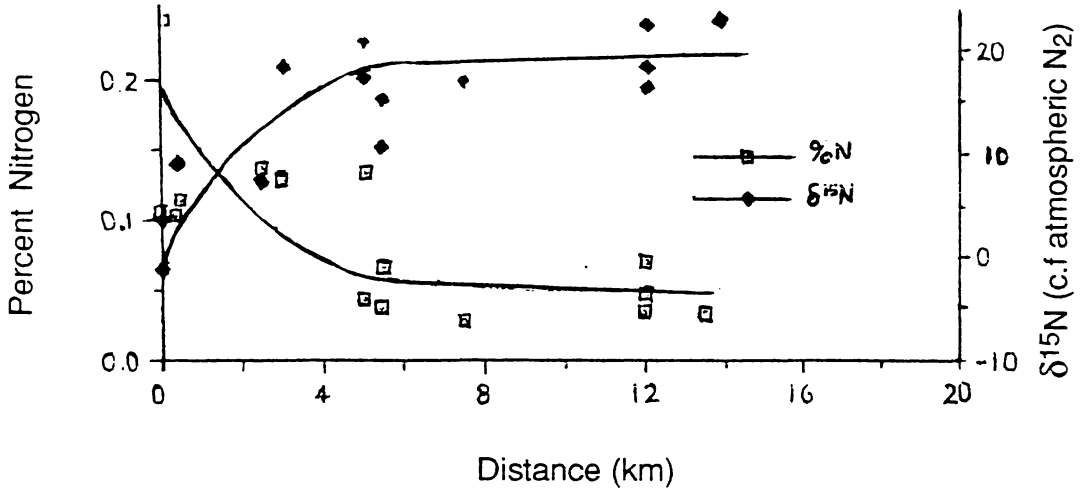


Figure 5.3 : Sediment ^{15}N abundance and percentage N - content in the Northern Manukau with respect to the distance from the Manukau Purification Works via the circulation channels (fig. 3.4).

SOM source:	kN%	Atom % ^{15}N	$\delta^{15}N$
Facultative Pond 4	7.58 + .15	.36388±.00007	- 6.6 ± 0.2
Onehunga	0.30 + .07	.36883±.00015	+ 6.9 ± 0.4
French Bay	0.26 + .02	.36835±.00011	+ 5.6 ± 0.3
Waiuku Channel	0.31 + .02	.36868±.00029	+ 6.5 ± 0.8
Puponga Point	0.28 + .01	.37018±.00011	+10.6 ± 0.3
Weymouth	0.30 + .02	.37070±.00015	+12.0 ± 0.4

Table 5.4 ^{15}N abundance and Nitrogen percent for Suspended Organic Matter in the Manukau Harbour and sewage treatment plant. Fig. 4 shows the site locations. The ^{15}N abundance is shown as Atom % and as a comparison with atmospheric dinitrogen (δ).

5.3.1 Tropic Levels

Plant and animal samples from different tropic levels were collected from the Little Muddy Creek site in the Manukau Harbour and processed by the tracermass method (section 2.4.3).

Table 5.5 shows that all the plant and animal samples are enriched, having a similar value to Gracillaria. There does not appear to be any tropic level enrichment.

Species	N%	Atom % ^{15}N	$\delta^{15}N$
Algae:			
Enteromorpha	2.82 ± .01	.37278±.00033	+17.69 ± .89
Gracillaria			
Red 1	3.09 ± .13	.37188±.00024	+15.23 ± .65
Red 2	0.92 ± .08	.37229±.00040	+16.4 ± 1.1
Filter Feeders:			
Perna	9.53 ± .56	.37070±.00034	+12.00 ± .93
Vermicularia	8.9 ± 1.8	.37047±.00037	+11.4 ± 1.0
Carnivores:			
Hemigrapsus			
- (flesh)	6.6 ± 1.2	.37165±.00020	+14.61 ± .55
- (eggs)	8.8 ± 1.4	.37135±.00046	+13.8 ± 1.3
Lunella	10.4 ± 1.2	.37168±.00010	+14.70 ± .27
Perinereis	9.39 ± .48	.37311±.00025	+18.59 ± .67

Table 5.5 : ^{15}N abundance and tissue Nitrogen percent for a preliminary study into tropic level ^{15}N abundance in the Manukau Harbour (Little Muddy Creek site).The ^{15}N abundance is shown as Atom % and as a comparison with atmospheric dinitrogen (δ). Three tropic levels are shown algae, filter feeders and carnivores. Fig. 4 shows the site location and appendix C2 gives the full species names.

Chapter 6

Modelling the impact of various NH_4 sources in a water body.

6.1 Preamble

The stable isotope data obtained for various N compounds (in a system) represent qualitative information which when combined with flow rates, may allow modelling of the entire N production and uptake (system). Quantitative data about water and nitrogen flow rates and source contributions to total N flow, enables the interpretation of an isotopic study in a water body, when it is combined together with the known effects of nitrogen processes (Section 1.4.2), and it is the purpose of this chapter to show how this has been done for a treated effluent discharge into the Upper Waikato River (UWR) and Northern Manukau Harbour (NMH).

The Upper Waikato River has been modelled here using the data of Huser (1989) for flow and N levels, along with isotope data from Chapter 4. Huser's study involved monitoring the various water sources to the UWR (ie. Taupo Gates outflow, Otumuheke, Waipouwerawera, Otumutaha and Huka Falls streams) over a 32 month period from January 1987 to August 1989 (Huser, 1989). A similar attempt has been made to model the NMH using existing flow data from Vant (1991).

When considering the Upper Waikato River system it will be necessary to make a number of assumptions regarding the system. The first is that the predominant source of N in the river water is NH_4 and that the small amount of NO_3 in the water does not significantly affect any outcomes. Above the sewage outfall the river is highly oligotrophic and surprising ca. 66% of the dissolved

inorganic N is NH_4. Immediately below the discharge, NH_4 accounts for ca. 85% of the total inorganic N (Miller, 1990).

The second assumption is that the river is fully mixed and that ammonium from an upstream source has an equal opportunity for uptake by plants at any point. This is patently not true as points immediately below a discharge are subject to a mixing zone. Despite this the assumption will hold for major reaches of the river.

The last assumption concerns the potential for *insitu* fractionation which is maximum when the amount of N assimilated is small with respect to the N supply. Conversely minimum fractionation is expressed when a high proportion of available substrate is assimilated. In the river inorganic ammonium is rapidly removed from the solution, therefore it is assumed that fractionation will be low. However within the clarity pond at the Taupo pollution control plant (effluent source) the quantity of *insitu* ammonium removed by algae is small with respect to the total amount available, hence the maximum fractionation would be expected to be expressed during uptake.

In assessing the Northern Manukau Harbour the assumptions regarding the Upper Waikato River (UWR) system (predominant N source, N mixing and potential insitu fractionation) need to be adapted and restated for this system. The first assumption is that the predominant source of N in the harbour water is NH_4 and that any NO_3 in the water does not significantly affect any outcomes. In the harbour the N is derived from two main sources, oceanic, mostly NO_3, and the effluent water, predominantly NH_4 and concentrated near the sewage discharge. Thus near the sewage outfall in the Manukau Harbour this assumption is most likely true, however with more distant regions NO_3 would be expected to be a major source of inorganic N and therefore will be expected to have a significant impact on the plant uptake system in the other area of the harbour.

The second assumption is that the ammonium in the northern harbour is predominantly derived from the effluent discharge, and is circulated mainly in

the Purakau and Wairopa Channels (Section 3.3) with rapid dilution. In the Manukau Harbour several streams run into the harbour which would be expected to contribute a small amount of ammonium, although this will not be expected to significantly change the isotope ratio in the northern harbour.

The last assumption concerns the potential for insitu fractionation in the harbour, the basic theory has been restated above for the Upper Waikato River and will not be repeated here. In the Manukau treatment ponds the maximum isotope effect during plant uptake will be expected to be expressed, due to the high concentration of NH_4 but low removal by algae. Similarly immediately outside the outlet to the ponds in the harbour maximum fractionation will occur. Further away from the ponds the isotope effect will be low, due to high uptake relative to NH_4 levels.

6.2 Upper Waikato River System

6.2.1 NH_4 - N Inputs

The source flow rates, ammonia concentration and the ammonium discharge rates for various water bodies, at their point of discharge into the Upper Waikato River is shown (Table 6.1). Values for sites downstream of the Huka falls were obtained by an extrapolation of Huser's data and are similar to values collected during this study. The major sources of ammonium in the river are: the outflow of Lake Taupo (ca. 33% of total NH_4-N) and the discharge from the Taupo Borough Pollution Control Plant (TB - PCP) (ca. 64%of total NH_4-N), however the ammonium concentration of these two sources are quite different, the flow rate of water from Lake Taupo is high with a low ammonium concentration, compared to the TB - PCP discharge effluent which has a much higher concentration of ammonium. The other point sources in the Upper Waikato River streams, make only a small contribution to the total ammonium

flow with the Otumuheke stream being the largest other contributor (ca. 3%), hence they can be ignored. Groundwater would also be expected to contribute to the ammonium pool in the river, however this source is not included, because it is hard to determine accurately and is expected to have little effect on the outcome of this interpretation.

	Source Flow rates				
Ammonia source	Flow rate m^{-3} s^{-1}	%total	Ammonia - N mg m^{-3}	Ammonia - N mg s^{-1}	%total
Lake Taupo	155.500	99.73	4.1	637.55	33
TB - PCP	0.210	.13	5958.0	1251	64
Otumuheke Stream	0.115	.07	28.2	3.24	.2
Waipouwerawera Stream	0.036	.02	10.5	0.38	.02
Otumutaha Stream	0.022	.01	1633.2	58.80	3.0
Huka Falls Stream	0.035	.02	7.7	0.23	.01
Totals	155.9	100.00	-	1951	100.00

Table 6.1: Results from a 32 month survey (Huser, 1989) to determine the average flow rates of the Upper Waikato River water sources and their ammonium concentration, including the calculated average quantity of ammonium released per second. Flow rate is in cubic metres per second, ammonia concentration in milligrams per cubic metre and the ammonia contribution is in milligrams per second.

6.2.2 Plant NH_4 - N Uptake

In order to interpret the stable isotope study in the Upper Waikato River, the level of ammonium removal in each section needs to be assessed. This can be used to determine the magnitude of isotope discrimination expressed in the river. Table 6.2 shows the ammonium flow rates in the river for the sampling sites used in this study. Flow rates are similar (within one order of magnitude) for all the sites included in this study and range from 4.1 to 14.5 mg m^3. The specific ammonium removal rate can be calculated by multiplying the difference in ammonium levels between an upstream site and a downstream site by the flow rate and dividing by the distance between the upper and lower boundaries of the particular section of the river in question (eq. 6.1).

$$\text{Specific Removal Rate (mg } m^3 \text{ } min^{-1}) = \frac{[\Delta] \text{ (mg} m^3) \text{ x flow rate (m. } min.^{-1})}{\text{distance (m)}} \qquad \text{(Eq. 6.1)}$$

For example,to calculate the apparent removal rate in the river section between T5 (Site A) and Reid's farm (Site B). The ammonium flow is 10.85 mg m^3 in the upstream site and 9.95 mg m^3 in the downstream site, hence the difference in concentration between the sites is 10.85 - 9.95 = 0.95mg m^3. The average flow rate is 90 $m.min^{-1}$ and the distance between the upper and lower limits of the river section is 1km. Thus the specific removal rate is:

$$\text{Specific Removal Rate (mg } m^3 \text{ } min^{-1}) = \frac{[0.90] \text{ (mg } m^3) \text{ x 90 (m. } min.^{-1})}{1000 \text{ (m)}} = 0.08 \text{ mg } m^3 \text{ } min^{-1}$$

Site	T. G. NH_4 - N (mg m^3)	TB-PCP NH_4 - N (mg m^3)	Total NH_4 - N (mg m^3)
Taupo Gates	4.10	-	4.10
Below Outlet	4.09	8.04	12.13
Cherry Island	4.09	10.41	14.50
T5	3.66	7.19	10.85
Reid's Farm	3.37	6.58	9.95
Upper Huka	3.43	5.97	9.40
Lower Huka	3.36	5.84	9.20
Wairaki	2.98	5.17	8.15

Table 6.2: The concentration of total ammonia - N in the Upper Waikato River at various sites and the calculated proportion of the total ammonium - N pool derived from various point discharges into the Upper Waikato River. All values are expressed in milligrams of ammonium - N per cubic metre of water. The total ammonium - N values are calculated values from the study by Miller (1990).

The percentage specific removal rate of ammonium from the river can be calculated using the specific removal rate and the original level of NH_4 at the upper endpoint (ie. Site A) in the river section (eq. 6.2).

$$\text{\% Specific Removal Rate } (\% \text{ min}^{-1}) = \frac{\text{Specific Removal Rate (mg m}^3 \text{ min}^{-1}\text{)}}{\text{Site A [NH}_4\text{] (mg m}^3\text{)}} \times \frac{100}{1} \qquad \text{(Eq. 6.2)}$$

Using the previous example (for eq. 6.1) the percentage specific removal rate is calculated as follows:

$$\text{\% Specific Removal Rate} = \frac{0.08 \text{ (mg min}^{-1}\text{)}}{10.85 \text{ (mg m}^3\text{)}} \times \frac{100}{1} = 0.7 \text{ \% m}^3 \text{ min}^{-1}$$

The ammonium removal rates and the percentage removal rates have been calculated for all the sections in the Upper Waikato River (Table 6.3). In the Upstream site (ie Upstream - Below Outlet) the values are relatively low, this is due to the low plant biomass, while downstream the values are a reflection of a much higher biomass.

River Section	Specific Removal rate (mg NH_4 m^3 min^{-1})	% Specific Removal rate (%NH_4 removed) (m^3min^{-1})
Upstream-Below Outlet	0.08×10^{-2}	0.2×10^{-1}
Below Outlet - T5 site	0.10	0.8
T5 site - Reid's farm	0.08	0.7
Reid's farm-U. Huka	0.05	0.5
Upper - Lower Huka	0.06	0.6
Lower Huka-Wairaki	0.03	0.3

Table 6.3 : Calculated removal , and percentage removal rates of ammonium - N in various sections of the Upper Waikato River Water. The results are based on the ammonia concentration data presented in table 6.2., distances between sites and an average flow rate in the Upper Waikato River of 1.5 m sec^{-1} (Huser, 1989). The sites are shown in fig. 3.3.

6.2.3 Origin of N in Plants

In the Upper Waikato River (UWR) plant - N is derived from two sources (lake Taupo and Effluent). In the Upstream section of the river only Lake Taupo N is available thus all the nitrogen in a plant must derive from this source. However below the Taupo borough pollution control plant (TB-PCP) effluent is also available. To determine the contribution that each source makes to plant - N requires the use of a simple mass balance model.

The mass balance model uses the isotopic abundances of the sources (Table 6.4) and the product (ie. plant - N) to determine the proportion that each source contributes. This requires that the isotope abundances of the sources are different, and are known, along with abundance of plant - N and the magnitude of isotope fractionation during assimilation. The isotope abundance of effluent has been determined (Chapter 4) and is significantly enriched, plant - N has also been assessed (Chapter 5). Thus all these values can be used to solve two simultaneous equations (eq. 6.3 and 6.4) that determine the contribution from each source makes to the formation of plant nitrogen.

Equation 6.3 is a mathematical expression that asserts that the combining of the proportion of plant nitrogen derived from (a) source one and (b) source two must sum up to equal one. This equation requires no information on isotopic ratios or fractionation and is therefore solely an expression of total N (ie. total $N = {}^{14}N + {}^{15}N$).

$$1 = a + b \qquad \text{(eq. 6.3)}$$

a = source one

b = source two

For example if two sources contribute to the formation of a product and the amount that one source (a) contributes is known and is equal to 30% (proportion = .3) then the amount that is supplied by the other source (b) can be determined by using equation 6.3 (ie. b = 1 - .30 = .70).

If we now add in the ^{15}N abundances of the sources and the product to the previous equation (eq. 6.3) then the following expression is true (eq. 6.4). The ^{15}N abundance of nitrogen in a plant is equal to (a) the proportion of nitrogen from source (one) multiplied by (Atom % $^{15}N_A$) it's ^{15}N abundance, plus (b) the proportion of nitrogen from source (two) multiplied by (Atom % $^{15}N_B$) it's ^{15}N abundance.

$$\text{Atom \% } ^{15}N_p = a \text{ Atom \% } ^{15}N_A + b \text{ Atom \% } ^{15}N_B \qquad \text{(eq. 6.4)}$$

Where:

Atom % $^{15}N_p$ = The ^{15}N abundance of plant - N

Atom % $^{15}N_A$ = The ^{15}N abundance of source a ammonium

Atom % $^{15}N_B$ = The ^{15}N abundance of source b ammonium

For example: At the Below Outlet site in the Upper Waikato River *Enteromorpha nana* has the Atom % ^{15}N value of .36786 , the value for the the Lake Taupo N source is given by the Upstream Atom % ^{15}N value for Enteromorpha = .36727 (Source b), the value for the effluent source is .36920 Atom % ^{15}N (Source a) , thus:

$$.36836 = .36920a + .36727b \qquad \text{(eq. 6.4)}$$

$$.36727b = .36836 - .36920a$$

$b = 1.00297 - 1.00504a$ and $b = 1 - a$

therefore: $1 - a = 1.00297 - 1.00504a$

$297 = 504a$, $a = .57$ and by substitution in eq. 6.3 $b = 1 - .57 = .43$

Thus plant nitrogen derived from Lake Taupo ammonium is 43 % and from effluent 57 % for Enteromorpha below the outlet.

Further refining the model, fractionation can be taken into account. This can be modelled by converting the abundances of the substrates to the ratio that will be expressed in the product (eq 6.5).

$$\text{Atom \% } {}^{15}N_p = (a \text{ Atom \% } {}^{15}N_A \times \beta) + (b \text{ Atom \% } {}^{15}N_B \times \beta) \qquad \text{(eq. 6.5)}$$

Hence if we use $\beta = 0.999$ (which is a reasonable approximate for the maximum discrimination against ^{15}N in the river). The Lake Taupo source isotopic value already has the β effect included, hence an adjustment is only needed for the effluent source, therefore the example now becomes:

$.36727b = .36836 - .36920a\ (0.999)$

$b = 1.00297 - 1.00424a$ and $b = 1 - a$

therefore: $1 - a = 1.00297 - 1.00424a$

$297 = 424a$, $a = .70$ and by substitution in eq. 6.3 $b = 1 - .70 = .30$

Thus plant nitrogen derived from Lake Taupo ammonium is 30 % and from effluent 70 %, for Enteromorpha below the outlet.

Table 6.5 show the proportion of nitrogen in E. nana and L. major that is derived from Lake Taupo and Efluent N in the Upper Waikato River, using the mass balance model. Four distinct sections are present in the river : Upstream, Below Outlet - T5, Reid's farm - Wairaki and Aratiatia. The first section,upstream is unaffected by effluent ammonium, therefore all plant N comes from the Lake Taupo source.

Dissolved Ammonia Source	Atom % ^{15}N	$d^{15}N$
Lake Taupo (Enteromorpha)	.36727± .00020	+2.7 ± 0.6
Lake Taupo (Lagarosiphon)	.36674±.000	+1.20 ± 0.3
Taupo Borough Pollution Control Plant	.36920±.00015	+7.9 ± 0.4

Table 6.4 : ^{15}N in ammonia (expressed as atom % ^{15}N) from various sources in the Upper Waikato River and associated sites. The ^{15}N value for the Lake Taupo and Lake Taupo plus river sediments is given by the value for *Enteromorpha nana* and *Lagarosiphon major* respectively. The TB -PCP is a direct measurement of the dissolved ammonia in sewage in the clarity pond that is discharged into the Upper Waikato River.

	Enteromorpha		Lagarosiphon	
Site	Lake Taupo	Waste water	Lake Taupo	Waste water
Upstream	100	0	100	0
Below Outlet	28^{b} - 43^{a}	57^{a} - 72^{b}	58^{b} - 65^{a}	35^{a} - 42^{b}
Cherry Island	-	-	21 - 32	68 - 79
T5	49 - 58	42 - 51	-	-
Reid's Farm	0 - 18	82 - 101	-	-
Upper Huka	7 - 25	75 - 93	-	-
Lower Huka	16 - 32	68 - 84	9 - 23	77 - 91
Wairaki	0 - 20	80 - 110	0 - 0	103 - 121
Aratiatia	-	-	50 - 58	42 - 50

Table 6.5 : The calculated percentage of Nitrogen in *Enteromorpha nana* and *Lagarosiphon major* derived from Lake Taupo or waste water sourced NH_4 - N. (a) assumes that there is no fractionation (ie β = 1.000) and gives the maximum proportion of N from Lake Taupo and the minimum from effluent. (b) assumes maximum fractionation (ie β = .999) and gives the minimum values for N from Lake Taupo and the maximum from effluent. Sites are shown in fig. 3.3.

The second section, Below Outlet to T5 can be regarded as a mixing zone for the two nitrogen sources. In this section approximately 50% of the nitrogen in the plants (ie. E. nana and L. major) is from each source. The amount of nitrogen that is derived from effluent is lower than is predicted by ammonium flow rates in the river (Table 6.1) and is due to the lack of mixing (ie. close to effluent discharge point). Immediately above the Below Outlet site effluent is discharged midstream, across the bottom of the river, the effluent is at a different temperature to the river and would be expected to travel along the river's bottom, until complete mixing is achieved. Both E. nana and L. major (tips) tend to be in the upper part of the water column, hence the effective pool of ammonium surrounding a particular plant, that is available for uptake, will contain a lower proportion of effluent N than is present in the total water column.

The third section of the river is from Reid's Farm to Wairaki, in this section 70 - 100% of assimilated ammonium is from the effluent source. In this section the river would be expected to be mixed (due to it being several km downstream). The proportion of ammonium from the effluent source is higher than is predicted from the total flow, this may be due to diurnal fluctuations in the flow rate from Lake Taupo. When the flow rate is lowest it effectively means that the quantity of ammonium surrounding a particular plant that is available for uptake (in this section of the river) will be highest due to reduction in the dilution of effluent ammonium. Plants in the river are N limited, therefore when the amount of N is increased uptake will increase, hence during this time the removal rate by plants will be higher than at other times and the change in proportions will result in a larger amount of effluent N being represented in the plants tissues. In section two this effect will also occur, though it's impact on plant N will be reduced due to incomplete mixing and reduced availability of effluent ammonium (compared with section three).

The fourth section of the river is Aratiatia (or Lake Aratiatia), where approximately 50% of the ammonium in L. major is from effluent. The amount

of L. major N from the effluent source is lower than would be expected as sediment N probably plays an increasing role in plant nitrogen. The lake bottom acts as a sink for organic N washed downstream, this material will be mineralised in the sediment and become available to L. major which will be able to take it up through it's roots. E. nana (not collected in this section) is not rooted therefore it will not contain any sediment N the proportion of effluent nitrogen assimilated will be similar to section three.

6.3 Northern Manukau Harbour System

6.3.1 NH_4 - N Inputs

Vant's data (Table 6.6) shows the concentration of total N in the Northern Manukau Harbour (Vant, 1991). The values are the averages from a 12 month survey from four sites in the harbour (fig. 3.4). In the harbour it is expected that there will be a high concentration of N near the Manukau treatment plant with rapid dilution in the harbour near the treatment plant, decreasing it is carried away with the current. Site one and two have similar, high nitrogen concentrations, this is five to ten times greater than those found in other North Island coastal waters (Vant, unpublished). This is a reflection of the treatment plant's discharge into the harbour (c. 70 kg NH_4 - N hr^{-1}). Levels at site three and four are lower than at one and two, due to the rapid dilution of the effluent in the harbour, but are still quite high compared with other systems.

6.3.2 NH_4 - N Impact

The impact of the effluent ammonium on G. secundata and sediment nitrogen content in the Northern harbour can be assessed by examining their

percentage N composition and isotopic values in the harbour. G. secundata has been shown to absorb ammonium preferentially to nitrate (Johnson, unpublished) therefore the tissue N content should reflect the ammonium flow in the harbour.

Sediment N is primarily a mixture of various decaying materials, therefore the nitrogen would be expected to reflect the organisms land the environment they lived in. However as sediments are generally not stable, there is potential for material from outside the area to be introduced.

Using the concentration of nitrogen in the harbour to predict the effect on percent N and it's isotopic value in plant and sediments, it would be expected that near the treatment plant there will be a logarithmic relationship with respect to distance from the outlets becoming linear as the concentration is lowered.

Figures 5.2 and 5.3 shows the relationship between the percentage N - content and the isotopic values with respect to the distance from the Manukau treatment plant for G. secundata and sediments. For G. secundata (fig. 5.2) the slope of the lines indicate a linear relationship which contrasts the logarithmic phase predicted near the plant by nitrogen concentration in the water. Similar relationships occur in sediment N (fig. 5.3), here the slope of the lines are similar to that predicted. The contradiction that is apparent for G. secundata with the predicted effect in the harbour may be due to the relatively small number of data points obtained, with the slopes of the percentage N and isotopic value relationships on the sediment graph best indicating the interaction with ammonium concentration in the harbour.

The percentage N composition and isotopic values of sediment and G. secundata can be used to predict the area in the harbour where a sample of unknown origin came from. By determining the percentage N content and the isotopic value of a sample the values can be compared with the graphs (fig. 5.2 and 5.3) to determine the distance that the unknown location is from the treatment plant.

Site	Total - N (mg m^3)
1	1630
2	1320
3	710
4	260

Table 6.6 Flow rates of total - N in the Northern Manukau Harbour, from sites one to four (fig. 3.4) from a 12 month study by Vant (1991).

6.3.3 Origin of N in SOM

In the Manukau Harbour SOM nitrogen is derived from two sources: NH_4 (from the effluent) and NO_3 (from marine nitrification). In the Manukau treatment plant SOM comprizes mostly live algae which assimilate the ammonium in the waste water. In the southern harbour SOM is mostly dead organic material derived from a variety of sources that have utilised nitrate as their N source.

The origin of SOM in the harbour can be determined using the mass balance model (Section 6.2.3). The isotopic values from SOM (Table 5.4) can be incorporated into the model to determine the proportion of N that is derived from each source. The isotopic value obtained from the facultative pond sample, can be used to represent the value that is obtained when only ammonium is assimilated by the organisms, similarly the Weymouth sample can be used to represent the value when only nitrate is assimilated. These value are suitably different to model their significance in samples of mixed origin.

Table 6.7 shows the proportion of N in SOM that was derived from effluent and oceanic sources. The values obtained do not take into account the isotopic effect associated with ammonium assimilation in the northern harbour by the treatment plant sourced SOM as this is variable (as shown by G. secundata and sediments) and the N assimilated is not expected to contribute significantly to the overall N content due to the mobility and the relatively short retention time of the material in the harbour (compared with sediments and plant materials).

Site	Atom % ^{15}N	% Effluent N	% Oceanic N
Facultative Pond 4	.36388±.00007	100	0
Onehunga	.36883±.00015	27	73
French Bay	.36835±.00011	34	66
Waiuku Channel	.36868±.00029	29	71
Puponga Point	.37018±.00011	7	93
Weymouth	.37070±.00015	0	100

Table 6.7 : The calculated percentage of Nitrogen in SOM from effluent NH_4 or oceanic NO_3, and the isotopic values for samples collected from within the Manukau Harbour. Sites are shown in figure 3.4.

Chapter 7

Discussion

7.1 Preamble

The usefulness of N stable isotope methodology in tracing waste water ammonium, depends on it's natural abundance, as well as the fractionation that occurs in the receiving body. Several studies have reported ^{15}N enrichment in effluent NH_4 (Sweeney et al, 1980, Estep and Vigg, 1985, Halley, 1990 and Miller, 1990) however no work (to the knowledge of the author) has been carried out investigating the cause and potential use of this phenomenon in tracer studies. This project has been very much a preliminary study into evaluating the usefulness of this method in tracing effluent ammonium in the environment.

7.2 Effluent NH_4 ^{15}N enrichment

Treated waste water has been shown to be enriched in by +7 to +20 $^{o}/_{oo}$ above natural abundance (as shown in Table 4.2). Domestic effluents from the Taupo, Hamilton and Manukau treatment plants and industrial effluents from Horotiu and Te Aroha showed no obvious differences between their enrichment values. This suggests that the cause of the enrichment in effluent is not primarily (or solely) due to the source of the material but must be linked to it's processing.

Wastewater becomes progressively enriched as it passes through a treatment plant, this is well shown for Horotiu (Table 4.3a) the possible mechanisms for this have been addressed in chapter four. In view of the

Horotiu treatment plant these processes are revisited here. At the Horotiu treatment plant primary effluent from the nearby Affcco freezing works (which kills sheep and cattle) enters the plant with an enrichment of +4.1 $\delta^{15}N$ in it's soluble fraction, reflecting the value of the major dissolved constituents blood and faeces. The blood and faeces of cattle have been previously shown to have an enrichment above natural abundance of +2.3 to +4.3 $\delta^{15}N$ (Steele and Daniel, 1978). In this system fat particles are removed from the surface of the wastewater and sludge is scrapped from the bottom.

After primary treatment, the waste water passes into an anaerobic pond where bacteria reduce the BOD, breakdown disease organisms present in the waste water and mineralise ammonium as indicated by the large increase in concentration (ie. from 12.7 in the primary to 106 mg NH_4 - N l^{-1} in the anaerobic pond). The newly mineralised ammonium is a new N source and must have an isotopic ratio similar to the overall value measured in the pond (ie. +10.7 $\delta^{15}N$) due to the apparently large contribution (c. 90%) it makes to the total NH_4 pool in the pond. The mineralised ammonium mixes in with the existing NH_4 to increase the isotopic enrichment of the dissolved pool. The actual isotopic value of the mineralised ammonium can be determined using the mass balance model (Chapter 6) although this is not necessary. The isotopic value of the newly mineralised ammonium is a reflection of it's source as the isotopic effect (β) during this process will be very low in the pond due to the high proportion of substrate that is mineralised and the low degree of isotope fractionation that occurs when most of the substrate is used up. This is supported by the study of Miyake and Wada (1971) who showed that there is little overall fractionation during bacterial mineralisation of organic N. The most likely substrate for mineralisation is animal protein that constitute part of the waste water. Gaebler et al (1966) showed that rat proteins are enriched by +5.2 to +12.6 $\delta^{15}N$ and similar values are probably found in sheep and cattle. The higher isotopic value of ammonium derived from protein will increase the overall pond enrichment.

Following on from the anaerobic treatment in the Horotiu treatment plant, waste water passes into a facultative pond where a variety of processes interact with the ammonium to increase enrichment to 13.3 $\delta^{15}N$. The main processes that use NH_4 as a substrate are nitrification, immobilisation, assimilation and volatilisation. Isotope fractionation associated with these processes would be expected to be near the maximum possible due to the relatively small quantity of ammonium that is apparently processed with respect to the total amount available. The highest fractionation would be expected to occur during nitrification as this process has been shown to produce the largest isotopic effect (besides denitrification). Mariotti et al (1981) and Mariotti (1982) observed a constant high isotopic effect of β equal to .9661 (this is equivalent to a 33.9 $^{o}/_{oo}$ depletion with respect to the substrate) during the oxidation of NH_4 to NO_2 by *Nitrosomonas europaea* in experiments where the quantity of ammonium substrate was maintained at a very high level with respect to the amount that was nitrified. When the quantity of ammonium was allowed to decrease significantly the isotopic effect decreased (ie. β increased). The facultative ponds mimic the experimental system of Mariotti et al (1981) and Mariotti (1982), the quantity of ammonium in the waste water is high with respect to the amount that undergoes nitrification. The constant flow replenishing of the system ensures that the amount of ammonium present remains high and therefore the isotopic effect will be maintained at it's maximum value. The actual isotopic effect in the facultative pond is unable to be determined due other processes using the NO_2 formed as a substrate but is likely to have a significant impact on NH_4 enrichment.

Immobilisation of NH_4 is a second process that may be significant in the facultative pond. The degree that the waste water is fractionated during immobilisation is hard to predict due to the complexity of the N exchange with sediments, nitrogen is added to the soil via the immobilisation pathway and is removed through mineralisation (Shearer and Kohl, 1986). The processes

that are involved in immobilisation are not the reversed order for mineralisation pathway, and therefore their isotopic effects are likely to be different and the measurement of their actual individual isotope effects difficult to carry out. Immobilisation is unlikely to have a significant impact on NH_4 enrichment within the pond.

The third process which is present in the facultative pond is algal assimilation of *insitu* ammonium, this has been shown (in Table 4.4) to result varying degrees of fractionation (β ranges from .9781 to .9932, which is equal to a 21.9 to 6.8 $^{o}/_{oo}$ depletion). Isotopic fractionation associated with this process can be quite considerable (as shown by the low β value) however the average isotopic effect measured was approximately β = .9860 (14 $^{o}/_{oo}$ depletion) for the systems used in this study. The total amount of N that is contained within algal tissue in the pond is relatively low (<5%) compared with the total N in the pond. The effect of assimilation on the enrichment of the waste water ammonium can be shown using the mass balance model (chapter 6). If we assume that the total algal nitrogen (=5%) is assimilated within the time it takes for a complete turnover of the waste water in the facultative pond, with an average isotopic effect of β = .9860 then the overall effect on the total NH_4 in the pond is to increase enrichment by 0.7 $\delta^{15}N$ with respect to the original enrichment of the waste water. This residence time in the facultative pond is approximately seven days, the amount of assimilation will clearly be much less than was used in the previous example, thus this process is not expected to contribute significantly to the enrichment of the ammonium in the pond.

The fourth major process that occurs in the facultative pond is NH_3 volatilisation, this process has been previously reviewed in chapter 1, the fractionation associated with the overall process has been examined in the pond. Ammonium in the pond is present as the neutral molecule (NH_3) and the ionised form (NH_4) with the ratio of the two forms primarily dependent on the pH of the solution (Suzuki et al, 1974). The pH was measured in the pond

at the beginning and end of a period when atmospheric ammonia was collected above the ponds and found to be approximately pH 8. A pH of 8 indicates that about 10% of the ammonium is in the form NH_3 and may be volatilised (Freney and Simpson, 1981). Two methods (measuring cylinder trap and acid tray) were compared for their apparent β values for the NH_3 volatilisation pathway, the methods are described in chapter 3. The isotopic effect measured by the cylinder traps varied between β = .9586 to 9806 depending on concentration in the atmosphere (fig 4.1) this is a depletion in ^{15}N by 41.4 to 19.4 $^{o}/_{oo}$ with respect to the substrate. The isotopic effect observed using the trays showed an even greater effect (average β = .9384 or a depletion of 61.6 $^{o}/_{oo}$). The maximum isotopic effects determined by the two methods (41.4 and 61.6 $^{o}/_{oo}$ depletion) are both greater than the maximum value of 34$^{o}/_{oo}$ predicted by theory for the NH_3 equilibrium reaction (Urey, 1947) and may indicate other fractionation steps in the process (eg. diffusion through solution) and / or in the collection methods (eg.diffusion into the phosphoric acid). Overall the fractionation associated with NH_3 volatilisation appears to be quite high and warrants further investigations, the process has the potential to be a major contributor to the enrichment of waste water NH_4 depending on the pH which is highly variable in treatment systems (Schroeder, 1981).

7.3 Plant ^{15}N abundance as an indicator of N source

Plants assimilate both nitrate and ammonium forms of N, some are also able to use atmospheric dintrogen through symbiotic relationships with bacteria (ie legumes and actinorhizal plants). The total plant nitrogen is a combination of the various forms that are absorbed and assimilated by the plants. An investigation into the ^{15}N abundance of plant - N, can be useful as an indicator of the N source and it's metabolism within plant cells. Numerous studies (eg. Rennie et al, 1976; Virgina and Delwiche, 1983; Estep and Vigg,

1985 and Mangawa and Wada, 1984) have shown that the ^{15}N values of plant N generally lie within the range of -5 to +10 $\delta^{15}N$ (compared with atmospheric N_2). Plants that utilise fixed N_2 have isotopic values similar to the natural abundance of their source. Other plants that assimilate inorganic N (NH_4 and NO_3) have isotopic values that cover the entire range (ie -5 to +10 $\delta^{15}N$) this is a reflection upon the variations in their N source and isotope fractionation during uptake and metabolism in plant cells.

Most ^{15}N studies of plant - N have been focused on terrestrial environments and in particular the importance of N_2 fixation by legumes, this is due to the economic value placed on this reaction in agriculture. Comparisons between the ^{15}N abundance of legumes and non - legumes (ie. non N fixers) has shown isotopic differences that have been used in the estimation of N_2 fixation by these plants (Shearer and Kohl, 1987; Bergersen, 1980, Bergersen and Turner, 1983 and Ledgard, 1984). A problem in comparing N_2 fixers with non - fixers is the variability in the latter which has been attributed to the heterogenity of ^{15}N in soil profiles and differences in the depth of root structures between species (Shearer and Kohl, 1986). This variation between non - legumes has been resolved by the use of plants that have similar root structures to the legumes

In comparison to the terrestrial environment, ^{15}N natural abundance in aquatic plants has rarely been studied, most of what has is focused on marine phytoplankton and algae, the study in the Upper Waikato River (Chapter 5) is apparently the first isotopic comparison between freshwater plants. Thirteen plant species collected from the upstream section of the river (above the effluent discharge) had ^{15}N abundance values between -3.33 and + 3.36 $\delta^{15}N$ (Table 5.1). The variation in ^{15}N abundance in the river was considerably more than was expected, but may be explained by differences in N source utilisation, it's synthesis and metabolism by various plant species. Three N sources are present in the river (dissolved N_2, sediment and inorganic N) though not all are available to every plant species. Dissolved N_2

can be used by nitrogen fixers such as Nostoc and sediment N by plants rooted in the substrate (eg. *Lagarosiphon major*) and inorganic N by most of the species present. In most environments NO_3 is the dominating inorganic form of N, however in the river NH_4 comprises 66% in the upstream section and 85% downstream of the Taupo effluent discharge (Miller, 1990). Ammonium is readily taken up by plants and microbes (Vitousek and Matson, 1984 and Sprent, 1987) and in some plants may it may be a preferential N source. In other plants nitrate is critical in maintaining the cytoplasmic pH near neutrality so that organic acids may accumulate, without NO_3 uptake these species do not increase in biomass (Salsac et al, 1987). In the river it appears that L. major, *Elodea canadensis* and *Enteromorpha nana* are adapted for preferential NH_4 uptake due to their domination of the downstream section (Table 5.2) where the proportion of NH_4 in the inorganic pool is the highest (85%).

The synthesis and metabolism of nitrogen may result in an isotopic effect at each stage during the transformation of N within plant tissues and cells, variations in reaction rates between species could conceivably produce differences in overall ^{15}N abundances . Plant tissues have been shown to vary by up to 2 $^{o}/_{oo}$ (Shearer and Kohl, 1980a and Mariotti et al, 1980) nodules may differ from the rest of the plant by up to 10 $^{o}/_{oo}$ (Shearer and Kohl, 1986). Few studies have been carried out on the isotopic effect for reactions involving N within plant tissues and cells, those that have show considerable variation in β values. For example the isotopic effect for transamination reactions catalysed by glutamic oxalacetic transaminase has measured β values ranging from .9918 to .9983 (Macko et al, 1986). Amino acids synthesised by microorganisms have been found to a wide range of β values depending on their metabolic pathways (Macko et al, 1987). Phenylalanine (a component of protein) ammonia - lyase results in an isotopic effect of up to β = .9760 (Hermes et al, 1985).

The central factor in the use of natural abundance levels of ^{15}N in tracer studies of waste water impacts is whether fractionation during processes alters the isotopic abundance of nitrogen in the products and mask it's use a tracer of N source in a system. The answer to this question is critical to the interpretation of the Upper Waikato River system, especially in the upstream section where inorganic N concentration is very low. In the upstream section the uptake rate is 0.02 mg m^{-3} $min.^{-1}$ from a total concentration of 4.1 mg m^{-3}. One might predict from isotope theory that such a low uptake would be associated with high fractionation, however in this section plant biomass is very low and one would predict that specific uptakes at the plant surface will be very high resulting in significant local removal of N. Indirect support for this, is obtained in the downstream section where plant biomass is much greater and uptake is higher (0.3 - 0.8 mg NH_4 m^{-3} $min.^{-1}$). Isotopic values downstream indicate that little or no fractionation occurs during uptake and it is concluded that the plants are efficient scavengers of soluble NH_4 and their isotopic values are a reliable indication of their source.

The usefulness of the mass balance mixing model is dependent on the reliability of the isotopic values measured (or estimated) for the NH_4 sources and plant ^{15}N abundances. This is of particular importance in the Upper Waikato River system where we are dealing with an enrichment in waste water NH_4 of only 7.9 $\delta^{15}N$. Variability in the waste water NH_4 enrichment was examined from samples taken on two occasions and found to be different by only 0.2 $^{o}/_{oo}$. Whether this is a fair indication of the variation in this source it is unclear. The Manukau treatment plants waste water NH_4 was found to vary by 2.5 $^{o}/_{oo}$ between two samples collected at different time, however NH_4 enrichment in this system is much higher (+20 $\delta^{15}N$) and the large variation may be due to a higher fluctuation in process fractionation. The Lake Taupo NH_4 source would not be expected to remain constant. Studies by Hoering (1957), Pang and Nriagu (1977) and Mariotti et al (1984) have all shown that natural sources of NH_4 may vary over time. If there is a considerable variation

in the Lake Taupo source it will affect the ^{15}N abundances of both upstream and downstream plants in the river and will be averaged out. So long as a difference is maintained between the two sources overall, variations will have little effect on the modelling of the origin of plant N. The second potential error results from plant ^{15}N abundance variations, this has been found to be up to 1.8 $^{o}/_{oo}$ for L. major and E. canadensis in the river, with most values being around 1.0 $^{o}/_{oo}$. The variation is quite large, considering we are dealing with an overall enrichment in the waste water of +7.9 $\delta^{15}N$. To minimise this effect on the modelling of N source, average enrichments were used for L. major at each site, unfortunately this was not possible with E. nana as this was not collected in replicates at the same sites. Thus the values for the origin of nitrogen in L. major can be considered to be more accurate than for E. nana. It is noted that the values predicted for the Wairaki site appear to over estimate the waste water NH_4 portion of plant N, this may be partially explained by it's distance downstream from the waste water discharge and the compounding of errors and secondly it may be an appreviation of the site (eg. presence of significant geothermal NH_4 source ?). The actual values for plant N, from each NH_4 at each site in the river source is generally expected to lie within the range of values determined.

In the Northern Manukau Harbour, the maximum variation in the isotopic effect associated with NH_4 assimilation is apparent, this has proved to be useful in determining source mixing (such as SOM) and can be used in determining whether NH_4 is in excess supply with respect to assimilation in the northern harbour. Fractionation near the Manukau treatment plant is maximised during assimilation and results in low ^{15}N values (-1 to +7 $\delta^{15}N$) compared with the waste water NH_4 (c. +20 $\delta^{15}N$). These values are similar for algae in the pond and would seem to indicate that there is an excess of NH_4 near the treatment plant. Sites further away from the treatment plant, have ^{15}N abundance values nearer to that of waste water (ie. +12 to +14.3 $\delta^{15}N$) and may be though to reflect an increase in enrichment of the inorganic

ammonium in the harbour due to fractionation during assimilation near the source. However plant productivity in the harbour is relatively low and would not be expected to remove enough N to affect the enrichment. The major effect in the harbour is dilution, therefore the ^{15}N abundances of plants from sites that are more distant reflect the less plentiful NH_4 and their greater uptake and efficiency at reducing the local NH_4 pool available to them.

Appendix A1 :

Measured δ values in parts per thousand ^{15}N ($^{o}/_{oo}$ ^{15}N) for the minerals Pitchblend and Urainite obtained from various sources.

$\delta^{15}N$ Range (in $^{o}/_{oo}$)	Reference
<u>Pitchblend</u>	
-2.3	Hoering (1955)
+46.4 to +61.3	White and Yagoda (1950)
-7.2 to +0.3	Scalan (1959)
-3 to +3	Pilot (1963)
<u>Urainite</u>	
+49.1, +67.6	White and Yagoda (1950)
+953.0	Hauck (1973)
<u>Cordierite</u>	
+4.3 to +5.9	Scalan (1959)

Appendix A2 :

Measured δ values in parts per thousand ^{15}N ($^{o}/_{oo}{}^{15}N$) for the organic fuels crude oil, gas, coal and peat obtained from various sources.

δ ^{15}N Range (in $^{o}/_{oo}$)	Reference
<u>Crude Oil</u>	
-11.5 to +2.9	Hoering (1955)
+14.2 to +16.9	Smith and Hudson (1951)
+2.3 to +5.1	Wada et al (1975)
<u>Natural Gas</u>	
-13.0 to +2.9	Hoering (1955)
+2 to +18	Bokhoven and Theeuwen (1966)
<u>Coal</u>	
-1.4 to +1.6	Hoering (1955)
-5.4 to +11.5	Parwel et al (1957)
+6.6 to +21.0	Smith and Hudson (1951)
-3.3 to - 2.2	Pilot (1963)
<u>Peat</u>	
-2.8 to +1.9	Hoering (1955)

Appendix A3 :

Measured δ values in parts per thousand ^{15}N ($^{o}/_{oo}{}^{15}N$) for particulate matter collected from a variety of different sites and type.

δ ^{15}N Range (in $^{o}/_{oo}$)	Reference
Soils	
-3 to +18	Bremner et al (1966)
-4 to +3	Bremner and Tabatabai (1973)
-1 to +17	Cheng et al (1964)
+0.9 to +4.2	Craft et al (1988)
+2 to +11	Delwiche and Steyn (1970)
+2.6 to +6.8	Ledgard et al (1987)
+4.6 to +10.2	Rennie et al (1976)
-7 to +6	Riga et al (1971)
+3.0 to +12.7	Shearer et al (1978)
+4.6 to +8.5	Wada et al (1975)
Sediments	
+6.1 to +8.1	Cifuentes et al (1988)
+2.9 to +13.4	Miyake and Wada (1967)
+3.9 to +5.0	Pang and Nriagu (1977)
+1.8 to +10.6	Sweeney et al (1980)
+3.1 to +10.5	Wada et al (1975)
Suspended Organic matter	
+7.7 to +10.3	Cifuentes et al (1988)
-0.9 to 7.5	Macko et al (1984)
+1.4 to +11.8	Mariotti et al (1984)
+2.3, +5.0	Owens (1985a)
+2.1 to +10.1	Saino and Hattori (1980)
-1.7 to +9.7	Wada and Hattori (1975)

Appendix A4 :

Measured δ values in parts per thousand ^{15}N ($^{o}/_{oo}{}^{15}N$) for a variety of N-containing liquids.

$\delta\ ^{15}N$ Range (in $^{o}/_{oo}$)	Reference
<u>Sewage:</u>	
+2.0 to +3.0	Sweeney et al (1980)
+9.0	Estep and Vigg(1985)
<u>Dissolved N_2</u>:	
+0.13 to +1.5	Benson and Parker (1961)
-0.9 to +2.3	Miyake and Wada (1967)
+0.21 to +1.30	Richards and Benson (1961)
<u>Dissolved NO_3</u>:	
+4.8 to +18.8	Cline and Kaplan (1975)
-7.2 to +3.4	Hoering (1957)
+5.1 to +7.5	Miyake and Wada (1967)
+5.05 to +6.55	Wada et al (1975)
<u>Dissolved NH_3</u>:	
-0.1 to +9.0	Hoering (1957)
+11.5 to +20.2	Horrigan et al (1990)
+8.2 to +12.5	Sweeney and Kaplan (1980a)
+10.0 to +29.0	Mariotti et al (1984)
-3.5 to +7.5	Miyake and Wada (1967)
+10.0	Wada and Hattori (1976)

Appendix A5 :

Measured δ values in parts per thousand ^{15}N ($^{o}/_{oo}{}^{15}N$) for non - vascular and vascular plants of different species and environments.

δ ^{15}N Range (in $^{o}/_{oo}$)	Reference
Nonvascular (algae)	
+2.9 to +8.6	Estep and Vigg (1985)
+7.9 to +8.1	Macko et al (1982)
+3.4 to +7.6	Minagawa and Wada (1984)
+5.8 to +9.7	Miyake and Wada (1967)
Vascular	
-4.1 to -2.9	Craft et al (1988)
-6.5 to +6.2	Hoering (1955)
-0.4 to +2.8	Hogberg (1990 a and b)
-5.0 to -2.2	Parwel et al (1957)
+0.7 to +4.2	Rennie et al (1976)
+4.0 to +12.7	Schulze et al (1991)
-7.6 to +17.5	Virginia and Delwiche (1982)
-1.0 to +5.9	Wada et al (1975)

Appendix A6 :

Measured δ values in parts per thousand ^{15}N ($^{o}/_{oo}{}^{15}N$) for a variety of animals comprising a mixture of species.

$\delta\ ^{15}N$ Range (in $^{o}/_{oo}$)	Reference
<u>Zooplankton:</u>	
+6.5 to +11.2	Estep and Vigg (1985)
+5.9 to +8.9	Macko et al (1984)
+9.6 to +12.8	Miyake and Wada (1967)
<u>Fish:</u>	
+8.6 to +16.2	Estep and Vigg (1985)
+8.4 to +12.9	Macko et al (1984)
+9.9 to +20.5	Miyake and Wada (1967)
+3.9 to +16.0	Schoeninger and Deniro (1984)
<u>Mammals:</u>	
+4.2 to +7.5	Hoering (1955)
+7.6 to +10.2	Macko (1982)
+1.9 to +23.0	Schoeninger and Deniro (1984)
+6.8 to +8.8	Wada et al (1975)

Appendix B1 :

Digestion Mixture

Chemicals required:

1l conc H_2SO_4 (AR grade)

100 g K_2SO_4 (elevates the boiling point about 10 °C)

1g SeO_2

Method :

Add K_2SO_4 and SeO_2 to acid then heat for three hours at 280 °C - 300 °C until cl. (The initial solution is green/black). Store in ca. 30 °C oven.

Appendix B2 :

Method for making 10M NaOH

Require 400g Naoh pellets / litre (Wear gloves and glasses at all times)

Materials:

NaOH pellets

2L plastic jug

Container to hold water

1.Weigh out 800 g of NaOH pellets in a large plastic weighing tray.

2.Transfer the pellets to a 2L plastic jug and fill with distilled water to about 1.9L.

3.When all the pellets are dissolved fill to the 2L mark and leave to cool before transfering to the reagent bottle.

Appendix B3 :

Method for making Mixed Indicator Solution

Require :

50ml mixed indicator (in reagent bottle-red colour) / litre

Materials:

Mixed indicator
2L conical flask
100ml measuring cylinder
1L measuring cylinder

1.Add 100 ml of mixed indicator to 1.9 L of distilled water

2.Adjust pH to 4.5 using a pH meter and 1M and 0.1M NaOH and HCl.

3.Fill to 2L, final solution is a 5% Mixed Indicator Solution.

Appendix C1: Insitu Waste water Algae Species List

Taupo Borough Pollution Control Plant

Clarity pond : Sample A. *Chlorococcum*

Sample B. *Chlorococcum*
Oedogonium
Oscillatoria
Desmids

Mangere Purification Works

Lagoon 4 (15 /1/91) : *Chlorogonium*
Chlamydomas
Chroococcus

Lagoon 4 (12/2/91) : *Chlorella*
Scenedesmus
Spirogyra

Pond 2 : *Chlorococcum*

Pond 4 : *Euglena*
Oscillatoria

Appendix C2 : Species list for samples collected in the Manakau Harbour

Algal Periphyton

Enteromorpha prolifera (Muell.) J. Ag.

Gracilaria secundata f. pseudoflagellifera Harv.

Ulva lactuca var. rigida (Ag.) Le Jol.

Animals:

Filter Feeders

Perna canaliculus Gmelin.

Vermicularia zelandicus

Carnivores

Perinereis novae-hollandie

Lunella smaragda Gmelin.

Hemigrapsus edwardsi

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