Use of Nuclear Techniques in Studies of Soil–Plant Relationships
USE OF NUCLEAR TECHNIQUES
IN STUDIES OF
SOIL–PLANT RELATIONSHIPS

EDITED BY
G. HARDARSON

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1990
FOREWORD

Isotope and radiation methods have proved to be very useful in agricultural research and in increasing world food production to the level at which it is today. These methods are being used routinely in fields like plant nutrition and soil fertility, plant breeding, animal production and health, insect pest control, food preservation and pesticide residue studies. With continuous improvements of isotope and radiation methods there is the need to update at regular intervals information used for the training of agricultural scientists in these fields.

The major limitation facing researchers in the developing countries when trying to benefit from the use of isotope and radiation methods for solving problems in their countries is the lack of technical information. Training courses are very efficient for transferring technology to the developing countries. The Soil Fertility, Irrigation and Crop Production Section of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and the Soil Science Unit of the IAEA Seibersdorf Laboratory have technical responsibility for organizing training courses on the use of isotopes in soil fertility and plant nutrition research. These courses have been held annually since 1978 until the present with two additional specialized courses conducted in the years 1985 and 1986 on biological nitrogen fixation. This manual was compiled from some of the lectures and practical exercises presented at these courses. It is hoped that it will be useful for future courses and for students or researchers working in these areas of research.
EDITORIAL NOTE

In preparing this material for the press, staff of the International Atomic Energy Agency have mounted and paginated the original manuscripts as submitted by the authors and given some attention to the presentation.

The views expressed in the papers, the statements made and the general style adopted are the responsibility of the named authors. The views do not necessarily reflect those of the governments of the Member States or organizations under whose auspices the manuscripts were produced.

The use in this book of particular designations of countries or territories does not imply any judgement by the publisher, the IAEA, as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.

The mention of specific companies or of their products or brand names does not imply any endorsement or recommendation on the part of the IAEA.

Authors are themselves responsible for obtaining the necessary permission to reproduce copyright material from other sources.
PREFACE

The Food and Agricultural Organization of the United Nations and the International Atomic Energy Agency established in 1964 a Joint FAO/IAEA Division, which is responsible for food and agricultural research and development involving nuclear techniques. The use of isotopes and radiation techniques in soil science and plant nutrition, plant breeding, animal health and production, insect pest control and food preservation have played an important role in increasing food production in the world.

Poorly fertile soils and low soil moisture are major constraints to plant productivity in the world. For economic reasons alone we have to help farmers in developing countries produce food as efficiently as possible in order to and to help in the constant battle against hunger with rising world population and to help conserve forests, which provide fuel wood - the developing world's major source of energy against excessive clearing. It is no surprise therefore that my colleagues in the Soil Fertility, Irrigation and Crop Production Section and the Soils Unit, IAEA Seibersdorf Laboratory have done considerable work developing nuclear techniques for the most efficient fertilizer strategy for plants, for an understanding of water requirements of crops (and the efficacy of irrigation methods) and for the enhancement of biological nitrogen fixation (thus relieving the farmer from having to buy expensive nitrogenous fertilizers). In all these studies isotope/nuclear techniques play a major and often unique role.

I am delighted that this manual is now available, replacing and updating earlier manuals (1964, 1976) and incorporating the vast experience of my colleagues in the matters above. The information it contains will surely help young scientists from the developing countries in the use of nuclear techniques in improving plant nutrition and therefore increasing food production in their countries. It is my hope that in the future we will be able to add to this impressive document by producing a manual in Spanish and special manuals focussing for example on root studies and the use of isotopes and related techniques.

G. BOWEN
Head of Soil Fertility, Irrigation and Crop Production Section
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
International Atomic Energy Agency
Vienna
CONTENTS

Stable and radioactive isotopes ................................................................. 9  
   H. Axmann, F. Zapata
Field experimentation in isotope-aided studies ........................................ 35  
   F. Zapata
Sample preparation techniques of biological material for isotope analysis .... 41  
   H. Axmann, A. Sebastianelli, J.L. Arrillaga
Methods for $^{15}$N determination ............................................................ 55  
   H. Axmann
Isotope techniques in soil fertility and plant nutrition studies ..................... 61  
   F. Zapata
Use of $^{15}$N methodology to assess biological nitrogen fixation ................ 129 
   G. Hardarson, S.K.A. Danso
Techniques in studies of photosynthesis .................................................. 161 
   K.S. Kumaranasinghe
Use of neutron water and gamma density gauges in soil water studies .......... 183 
   C. Kirda
INTRODUCTION

Almost all elements of importance in biological research have at least two stable isotopes, of which the heavier isotope (the one having atoms with higher mass number) occur naturally in small amounts. Heavy isotopes are often used as tracers in biological systems. The stable isotopes of the most common elements used in biological research are listed in Table 1.

Table 1: Stable isotopes used as tracers in biological research

| Element | Stable isotopes  | Heavy | Light
|---------|-----------------|-------|-------
| H       | $^2$D (0.0156%) |       | $^1$H (99.9844%) |
| N       | $^{15}$N (0.366%) |     | $^{14}$N (99.634%) |
| C       | $^{13}$C (1.108%) |     | $^{12}$C (98.892%) |
| S       | $^{36}$S (0.02%), $^{34}$S (4.22%), $^{33}$S (0.75%) |     | $^{32}$S (95.02%) |
| O       | $^{18}$O (0.204%), $^{17}$O (0.037%) |     | $^{16}$O (99.759%) |

In the above list, the values given in brackets are natural abundances. The stable isotope composition (ratio of light to heavy isotopes) of biological materials is measured either by mass spectrometry or optical emission spectrometry. The quantities of the heavier stable isotope in a sample are expressed in atom percent in excess of natural abundance. This will be explained in more detail in the present and the following chapters.

Early applications of the stable isotope analyses were made in the geological sciences such as geochemistry and cosmochemistry. Over the past few years, recent instrumental developments and innovative studies using
various stable isotopes have led to a widespread application in life sciences, agriculture and environmental research. Stable isotopes are thus providing valuable information about long term fluctuations in temperature, global CO$_2$ and precipitation and in areas of ecological research such as marine ecology, historical ecology and biogeochemical cycling. Also natural differences in the stable isotopic composition both between biotic and abiotic compounds and between biotic compounds derived from different biochemical processes are used as indicators of physiological processes as well as movement of compounds and energy through ecosystems. Therefore there is sufficient evidence that stable isotopes will lead to major advances in ecology and food-chain research.

**ISOTOPES OF NITROGEN**

Various radioactive and stable isotopes of N are known, with mass numbers from 12 to 17 (Table 2). The longest-lived radioactive isotope of N is $^{13}$N, with a rather short half life of only 10.05 minutes. This severely limits its use to agricultural research of very short duration. There are two stable isotopes of N, i.e. $^{14}$N and $^{15}$N.

<table>
<thead>
<tr>
<th>Mass number</th>
<th>Natural abundance (%)</th>
<th>Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>-</td>
<td>0.0126 s</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>10.05 m</td>
</tr>
<tr>
<td>14</td>
<td>99.634</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>0.366</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>7.36 s</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>4.14 s</td>
</tr>
</tbody>
</table>

The isotopic composition of $^{15}$N in the atmosphere is approximately 0.366 atom % of the total N in the atmosphere, while that of $^{14}$N is about 99.634 atom %. To every $^{15}$N atom in atmosphere are 272±0.3 $^{14}$N atoms yielding 0.3663±0.0004 atom % $^{15}$N.
The more or less constant ratio of $^{14}\text{N} / ^{15}\text{N}$ in the atmosphere or natural substances makes it possible to use N materials artificially enriched or depleted in $^{15}\text{N}$ as tracers in many studies. Since both $^{15}\text{N}$ and $^{14}\text{N}$ are not radioactive, the use of the $^{14}\text{N} / ^{15}\text{N}$ ratios in research does not involve health risks and hazards, and their stable nature allows long term experiments. In the past, the major factors which hindered the use of $^{15}\text{N}$ in agricultural investigations were: the high cost and maintenance problems associated with the instruments used for $^{14}\text{N} / ^{15}\text{N}$ ratio analysis, and the high cost of $^{15}\text{N}$ labelled materials. Both of these limitations no longer apply. A whole range of advanced instrumentation is now available for $^{15}\text{N}$ determinations with increased accuracy and precision. The cost of $^{15}\text{N}$ is now a minor part of the expenses involved in well-designed field experiments due to large demand for $^{15}\text{N}$ labelled materials.

$^{15}\text{N}$ tracer techniques in nitrogen research

$^{15}\text{N}$ has become an isotope of broad application for the understanding of biological and/or chemical processes affecting the nitrogen cycle and the movement of N compounds in agricultural systems. For detailed information regarding $^{15}\text{N}$ applications in agriculture and medicine, please refer to the reports of Faust (1981, 1983, 1986).

Within the FAO/IAEA programmes dealing with nuclear applications in agriculture, $^{15}\text{N}$ has been extensively used as a tracer in the following studies on soil-plant systems:

1) Nitrogen turnover in soil.
2) Distribution of applied N in soil organic matter.
3) Genotypic differences in nitrogen uptake and use.
4) Fertilizer N utilization:
   - Fertilizer management practices (timing, placement, sources, etc.).
   - Interactions with another agronomic factors (irrigation, plant species, cultivars, land preparation, etc.).
   - Fertilizer N balance.
5) Recovery of N from crop residues.
6) Nitrogen movement in soils.
7) Nitrogen gaseous losses (volatilization, denitrification, etc.).
8) Nitrogen leaching losses.
9) Environmental aspects of nitrogen use.
10) Degradation of organic chemicals added to soils.
11) Biological nitrogen fixation e.g. identification and comparison of N-fixing systems, measurement of biological nitrogen fixation in the field, improvement of biological nitrogen fixation.

12) Distribution of N among plant parts.

13) N metabolic studies in plants and animals.

Basic Nitrogen Isotope Terminology and Stoichiometry

$^{15}$N isotope terminology

The purpose of this section is to give some guidelines for the calculation of $^{15}$N data (Table 3). The symbols used are based on SI (Système International d'Unités) units.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Unit</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>$xl$</td>
<td>$^{15}$N abundance = the total atom % $^{15}$N in a sample.</td>
</tr>
<tr>
<td>$a_0$</td>
<td></td>
<td>natural abundance, atom % $^{15}$N natural abundance in nature is generally about 0.3663 atom % $^{15}$N + 0.0004</td>
</tr>
<tr>
<td>$\bar{a}$</td>
<td></td>
<td>mean $^{15}$N abundance</td>
</tr>
<tr>
<td>$a_1, a_2$</td>
<td></td>
<td>atom % $^{15}$N abundance of nitrogen in a compound 1, 2</td>
</tr>
<tr>
<td>$a_1', a_2'$</td>
<td></td>
<td>atom % $^{15}$N excess in a chemical compound (i.e. atom % $^{15}$N in excess of natural abundance)</td>
</tr>
</tbody>
</table>

$a_1' = a - a_0$
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Unit</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>g/mol</td>
<td>molar mass of a chemical compound (The sum of the atomic weights of all the atoms in a molecule).</td>
</tr>
<tr>
<td>( A_N, A_C, \ldots )</td>
<td></td>
<td>molar mass of an element</td>
</tr>
<tr>
<td>( A(14), A(15) )</td>
<td></td>
<td>molar mass of the nitrogen isotopes (^{14}\text{N}, \text{ }^{15}\text{N} )</td>
</tr>
<tr>
<td>( M_R )</td>
<td></td>
<td>molar mass of the compound without nitrogen</td>
</tr>
<tr>
<td>( M_{eq} )</td>
<td></td>
<td>molar mass of an equivalent (commonly called &quot;equivalent mass&quot;)</td>
</tr>
<tr>
<td>( n )</td>
<td>mol</td>
<td>amount of various entities such as chemical elements, compounds, equivalents, \ldots</td>
</tr>
<tr>
<td>( n_1, n_2, \ldots )</td>
<td></td>
<td>amount of compound 1, 2, \ldots</td>
</tr>
<tr>
<td>( n_N )</td>
<td></td>
<td>amount of nitrogen</td>
</tr>
<tr>
<td>( n_{N1}, n_{N2}, \ldots )</td>
<td></td>
<td>amount of nitrogen in compound 1, 2, \ldots</td>
</tr>
<tr>
<td>( n(15) )</td>
<td></td>
<td>(^{15}\text{N} ) amount</td>
</tr>
<tr>
<td>( n'(15) )</td>
<td></td>
<td>(^{15}\text{N} ) excess amount</td>
</tr>
<tr>
<td>( n(14) )</td>
<td></td>
<td>(^{14}\text{N} ) amount</td>
</tr>
<tr>
<td>( n'(14) )</td>
<td></td>
<td>(^{14}\text{N} ) excess amount</td>
</tr>
<tr>
<td>( n_{eq} )</td>
<td></td>
<td>amount of equivalents of chemical elements, compounds</td>
</tr>
<tr>
<td>( c )</td>
<td>mol/l</td>
<td>concentration of a chemical compound in a solution (molarity)</td>
</tr>
<tr>
<td>( c_{eq} )</td>
<td></td>
<td>normality</td>
</tr>
</tbody>
</table>

13
The relative isotope abundance of $^{15}$N in a particular chemical compound (e.g. N$_2$, urea) is defined by the ratio between the amount of the isotope $^{15}$N (basis SI unit = mol) and the amount of the total chemical nitrogen containing the isotope $^{14}$N and $^{15}$N (unit = mol). The unit of the relative isotope abundance of $^{15}$N is consequently 1 (mol/mol). This can be explained by the following equation:

$$\frac{a}{100} = \frac{n(15)}{n(14)+n(15)} = \frac{n(15)}{n_N}$$

Relative isotope abundance can be either expressed in:

1) $^{15}$N abundance (atom % $^{15}$N)

$$a = \frac{n(15)}{n(14)+n(15)} \times 100 = \frac{n(15)}{n_N} \times 100 \text{ (at. %)}$$

or

2) δ-values indicate small deviations per mil (‰) in isotope ratio of a sample related to that of a standard (usually atmospheric N).

$$\delta^{15}N = \left[ \frac{n(15)/n(14)}{[n(15)/n(14)] \text{ standard}} - 1 \right] \times 1000$$

The latter unit of isotopic ratio is mostly used for precision measurements in natural abundance range. Common standard is atmospheric nitrogen with an accepted value of $0.3663 \pm 0.0004$ at. % $^{15}$N.

The $\delta^{15}$N of biological materials can range from $-10\%$ (corresponding to 0.3626 atom % $^{15}$N) to $+15\%$ (0.3718 atom % $^{15}$N).

Stoichiometry

In order to calculate the equivalent amount of a chemical compound (e.g. fertilizers) for a given amount of nitrogen one has to know the formulae of the compound e.g. for ammonium sulphate = (NH$_4$)$_2$SO$_4$. The molecular weight or molar mass = M (in g/mol) can be calculated from the atomic weights.
of the elements forming the compound. \( M \) for \((NH_4)_2SO_4\) is therefore:

\[
\begin{align*}
2N &= 28.016 \\
8H &= 8.063 \\
S &= 32.064 \\
40 &= 63.997 \\
\hline
132.140 \text{ g/mol} &= M
\end{align*}
\]

1 mol \((NH_4)_2SO_4\) (= 132.14 g) contains 2N (= 28.016 g)

**Exercise 1:**

How much \((NH_4)_2SO_4\) is needed to apply 10 g N/m\(^2\) in a field experiment?

\[
M(NH_4)_2SO_4 = 132.14 \text{ g} \\
132.14 \text{ g } (NH_4)_2SO_4 \quad \text{-------} \quad 28.016 \text{ g N} \\
X \quad \text{-------} \quad 10 \text{ g N}
\]

\[
X = \frac{132.14 \times 10}{28.016} = 47.16 \text{ g } (NH_4)_2SO_4/\text{m}^2
\]

which is equivalent to 10 g N/m\(^2\).

**Exercise 2:**

The molar mass \((M)\) given in Table 4 is only valid for nitrogen compounds with \(^{15}\text{N}\) natural abundance \((a_o = 0.3663 \text{ at. } \% \ 15\text{N})\). \(^{15}\text{N}\) enriched compounds will have different \(A_N\), \(M\) and \(W_N\).

**Table 4:** Formulæ, \( M \) (molar mass) and \( \% \text{N} \) for the most common N-compounds used as fertilizer

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formulae</th>
<th>( M ) ( \text{g/mol} )</th>
<th>( % \text{N} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium sulphate</td>
<td>((NH_4)_2SO_4)</td>
<td>132.14</td>
<td>21.20</td>
</tr>
<tr>
<td>Urea</td>
<td>((NH_2)_2CO)</td>
<td>60.06</td>
<td>46.64</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>(NH_4Cl)</td>
<td>53.49</td>
<td>26.19</td>
</tr>
<tr>
<td>Ammonium nitrate</td>
<td>(NH_4NO_3)</td>
<td>80.04</td>
<td>35.00</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>(Na NO_3)</td>
<td>84.99</td>
<td>16.48</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>(KNO_3)</td>
<td>101.11</td>
<td>13.85</td>
</tr>
</tbody>
</table>
This exercise illustrates the effect of isotopic composition on i) \( A_N \) (average atomic weight of nitrogen), ii) \( M \) (molar mass) and iii) \( W_N \) (% nitrogen in a compound).

i) Calculate \( A_N \) for:

a) nitrogen with natural abundance of \( ^{15}N \) (\( a = 0.3663 \) at. \% \( ^{15}N \))

\[
^{15}N = \frac{a \times 15}{100} = \frac{0.3663 \times 15}{100} = 0.0549 \text{ g}
\]

\[
^{14}N = \frac{(100-a) \times 14}{100} = \frac{99.6337 \times 14}{100} = 13.9487 \text{ g}
\]

Total = \( ^{15}N \) + \( ^{14}N \) = 14.0036 g = \( A_N \)

b) nitrogen with 50 at. \% \( ^{15}N \) abundance = (\( a = 50.0 \) at. \% \( ^{15}N \))

\[
^{15}N = \frac{a \times 15}{100} = \frac{50 \times 15}{100} = 7.500 \text{ g}
\]

\[
^{14}N = \frac{(100-a) \times 14}{100} = \frac{50 \times 14}{100} = 7.000 \text{ g}
\]

Total = \( ^{15}N \) + \( ^{14}N \) = 14.5000 g = \( A_N \)

ii) Calculate \( M \) of \( (NH_4)_2SO_4 \) with:

a) natural abundance \( ^{15}N \) \( a = 0.3663 \)

\[
\begin{align*}
A_N &= 14.0036 \text{ g, } 2A_N = 28.0072 \text{ g} \\
8H &= 8.0632 \\
1S &= 32.0640 \\
4O &= 63.9976 \\
\hline
\text{Total} &= 104.13
\end{align*}
\]

\[
2N = 28.0072
\]

\[
\text{Total} = 132.14 \text{ g} = M
\]
b) 50 at. % \textsuperscript{15}N abundance \( a = 50.0 \)

\[ A_N = 14.5 \text{ g, } 2A_N = 29.0 \text{ g} \]

\[ \begin{array}{ll}
8H & 8.0632 \\
1S & 32.0640 \\
40 & 63.9976 \\
\hline
\end{array} \]

\[ \text{Total} = 104.13 \]

\[ 2N = 29.00 \]

\[ \text{Total} = 133.13 \text{ g} = M \]

iii) Calculate \( W_N \) = % nitrogen in \((NH_4)_2SO_4\) with:

a) natural abundance of \textsuperscript{15}N = \( a_o \)

\[ M = 132.14 \text{ g, } 2A_N = 28.0072 \text{ g} \]

\[ \begin{array}{ll}
132.14 \text{ g } (NH_4)_2SO_4 & \text{----- 28.0072 g nitrogen} \\
100 \text{ g } (NH_4)_2SO_4 & \text{----- X g nitrogen} \\
\hline
\end{array} \]

\[ \% N = \frac{100 \times 28.0072}{132.14} = 21.20 \]

b) 50 at. % \textsuperscript{15}N = \( a \)

\[ M = 133.13 \text{ g, } 2A_N = 29.0 \]

\[ \begin{array}{ll}
133.13 \text{ g } (NH_4)_2SO_4 & \text{----- 29.0 g nitrogen} \\
100 \text{ g } (NH_4)_2SO_4 & \text{----- X} \\
\hline
\end{array} \]

\[ \% N = \frac{100 \times 29}{133.13} = 21.78 \]

Exercise 3:

Calculate how many grams of \textsuperscript{15}N are in 100 g of \((NH_4)_2SO_4\) with:

a) \( a = 0.3663 \) at % \textsuperscript{15}N abundance
From previous exercises:

1 atom N = 0.0549 g $^{15}$N, 1 mol (NH$_4$)$_2$SO$_4$ has 2 atom N = 0.1098 g $^{15}$N

M = 132.14 (see Exercise 2iia)

132.14 g ------ 0.1098 g $^{15}$N

100.00 g ------ X

\[ g^{15}N = \frac{100 \times 0.1098}{132.14} = 0.083 \]

b) a = 50 at. % $^{15}$N abundance

1 atom N = 7.500 g $^{15}$N

2 atom N = 15.000 g $^{15}$N

and M = 133.13 g (See exercise 2iib)

133.13 ------ 15.00 g $^{15}$N

100.00 ------ X

\[ g^{15}N = \frac{100 \times 15.0}{133.13} = \frac{11.27}{11} \]

Exercise 4:

By how much is the $^{15}$N content of 50 at. % $^{15}$N abundance (NH$_4$)$_2$SO$_4$ underestimated if the calculation is wrongly based on the % N content of ordinary (NH$_4$)$_2$SO$_4$?

Calculation: 100 g ordinary (NH$_4$)$_2$SO$_4$ has 21.20 g N, 50% from this = 10.60 g $^{15}$N instead of 11.3 g as shown in Exercise 3b. Therefore, 11.3 g - 10.6 g = 0.7 g $^{15}$N is the difference due to underestimation or 6.2%.

Exercise 5:

Ordering of fertilizers: Based on the knowledge gained from the previous section and the exercises given below it is important to pay attention to whether the quotation of a firm selling $^{15}$N labelled fertilizer is given in terms of at. % $^{15}$N abundance (= a) or at. % $^{15}$N excess (= a').
i) Two firms have submitted the following bids for 1000 g urea 1 at. % $^{15}N$

\[ \text{excess } (a' = 1.00 \text{ or } a = 1.366) : \]

**Firm I**

**Offers 1 g $^{15}N$ in excess of natural abundance \((a')\) for 160 $**

<table>
<thead>
<tr>
<th>Element</th>
<th>Amount</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>30 x 0.01366</td>
<td>0.41</td>
</tr>
<tr>
<td>H</td>
<td>28 x 0.98634</td>
<td>27.62</td>
</tr>
<tr>
<td>N</td>
<td>27.62</td>
<td>955 $</td>
</tr>
</tbody>
</table>

\[ \text{Total = 60.07 g urea} \]

\[ g^{15}N \text{ excess } a' = (a-a_o) \]

\[ a_o = 30 \times 0.00366 = 0.11 \]

\[ a' = 0.41 - 0.11 = 0.30 \]

\[ 60.07 \text{ g urea} ---- 0.30 \text{ g }^{15}N \]

\[ 1000 \text{ g urea} ---- a' \]

\[ a' = 5 \text{ g }^{15}N \]

\[ 5 \text{ g }^{15}N \times 160 $ = 800 $ \]

**Firm II**

**Offers 1 g $^{15}N$ [total g $^{15}N$ in compound \((a)\)] for 140 $**

<table>
<thead>
<tr>
<th>Element</th>
<th>Amount</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>30 x 0.01366</td>
<td>0.41</td>
</tr>
<tr>
<td>H</td>
<td>28 x 0.98634</td>
<td>27.62</td>
</tr>
<tr>
<td>N</td>
<td>27.62</td>
<td>683 $</td>
</tr>
</tbody>
</table>

\[ \text{Total = 60.07 = M} \]

\[ g^{15}N \text{ total } a = 0.41 \]

\[ a = 6.83 \text{ g }^{15}N \]

\[ 6.83 \text{ g }^{15}N \times 140 $ = 955 $ \]

ii) Another comparison of bid quotation for $^{15}N$ labelled materials.

**Firm I** offers:

a) 1000 g (NH$_4$)$_2$SO$_4$ with 20 at. % $^{15}N$ abundance \((= a)\) for 5000 US $

b) 1000 g (NH$_4$)$_2$SO$_4$ with 3 at. % $^{15}N$ abundance \((= a)\) for 700 US $
Firm II offers:

a) 1000 g \((\text{NH}_4)_2\text{SO}_4\) with 20 at \% \(^{15}\text{N}\) excess = (a') for 5000 US $

b) 1000 g \((\text{NH}_4)_2\text{SO}_4\) with 3 at \% \(^{15}\text{N}\) excess (= a') for 700 US $

How much does 1 g \(^{15}\text{N}\) cost in Firm I and Firm II?

\[
\text{Firm I (a)}\\
\text{a) } ^{15}\text{N} \times 30 \times 0.2 = 6.00 \text{ g}^{15}\text{N}\\
M = 132.52\\
132.52 \text{ g} \((\text{NH}_4)_2\text{SO}_4\) --- 6.00 g\(^{15}\text{N}\)\\
1000 \text{ g} \((\text{NH}_4)_2\text{SO}_4\) ----- X g\\
X = \frac{1000 \times 6.00}{132.52} = 45.28 \text{ g}^{15}\text{N}\\
\frac{5000 \text{ US } \$}{45.28 \text{ g}^{15}\text{N}} = 110.4 \text{ US } \$/1 \text{ g}^{15}\text{N}\\
\text{b) } ^{15}\text{N} = 30 \times 0.03 = 0.900 \text{ g}^{15}\text{N}\\
M = 132.18\\
132.18 \text{ g} --- 0.900 \text{ g}^{15}\text{N}\\
1000 \text{ g} ----- X g\\
X = \frac{900}{132.18} = 6.81 \text{ g}^{15}\text{N}\\
\frac{700 \text{ US } \$}{6.81 \text{ g}^{15}\text{N}} = 102.8 \text{ US } \$/1 \text{ g}^{15}\text{N}\\
\text{Firm II (a')}\\
\text{a) } ^{15}\text{N} \times 30 \times 0.20366 = 6.11 \text{ g}^{15}\text{N}\\
M = 132.52\\
132.52 \text{ g} \((\text{NH}_4)_2\text{SO}_4\) --- 6.11 g\(^{15}\text{N}\)\\
1000 \text{ g} \((\text{NH}_4)_2\text{SO}_4\) ----- Y g\\
Y = \frac{1000 \times 6.11}{132.52} = 46.11 \text{ g}^{15}\text{N}\\
\frac{5000 \text{ US } \$}{46.11 \text{ g}^{15}\text{N}} = 108.4 \text{ US } \$/1 \text{ g}^{15}\text{N}\\
\text{b) } ^{15}\text{N} = 30 \times 0.03366 = 1.010 \text{ g}^{15}\text{N}\\
\frac{700 \text{ US } \$}{6.81 \text{ g}^{15}\text{N}} = 91.6 \text{ US } \$/1 \text{ g}^{15}\text{N}\\

These two exercises demonstrate that for low enriched material it is especially important to pay attention whether the cost is based on at \% \(^{15}\text{N}\) abundance or \% \(^{15}\text{N}\) atom excess.
Table 5 presents the calculated amounts of $^{15}$N (g) in ammonium sulphate and urea for different $^{15}$N enrichments.

Table 5: $^{15}$N content (g) in urea and ammonium sulphate at various $^{15}$N enrichments

<table>
<thead>
<tr>
<th>$^{15}$N labelled compound with at % N excess (a')</th>
<th>Ammonium sulphate</th>
<th>Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>g $^{15}$N excess</td>
<td>total g $^{15}$N</td>
<td>g $^{15}$N excess</td>
</tr>
<tr>
<td>----------</td>
<td>----------------</td>
<td>----------</td>
</tr>
<tr>
<td>at % $^{15}$N excess (a')</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.27</td>
<td>3.10</td>
</tr>
<tr>
<td>2</td>
<td>4.45</td>
<td>5.37</td>
</tr>
<tr>
<td>3</td>
<td>6.81</td>
<td>7.64</td>
</tr>
<tr>
<td>4</td>
<td>9.08</td>
<td>9.91</td>
</tr>
<tr>
<td>5</td>
<td>11.34</td>
<td>12.17</td>
</tr>
<tr>
<td>6</td>
<td>13.61</td>
<td>14.44</td>
</tr>
<tr>
<td>10</td>
<td>22.67</td>
<td>23.50</td>
</tr>
<tr>
<td>20</td>
<td>45.27</td>
<td>46.10</td>
</tr>
<tr>
<td>at % $^{15}$N abundance (a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.44</td>
<td>2.27</td>
</tr>
<tr>
<td>2</td>
<td>3.71</td>
<td>4.54</td>
</tr>
<tr>
<td>3</td>
<td>5.98</td>
<td>6.81</td>
</tr>
<tr>
<td>4</td>
<td>8.25</td>
<td>9.08</td>
</tr>
<tr>
<td>5</td>
<td>10.51</td>
<td>11.34</td>
</tr>
<tr>
<td>6</td>
<td>12.78</td>
<td>13.61</td>
</tr>
<tr>
<td>10</td>
<td>21.84</td>
<td>22.67</td>
</tr>
<tr>
<td>20</td>
<td>44.45</td>
<td>45.28</td>
</tr>
</tbody>
</table>

**ISOTOPE DILUTION**

**Principles and Equations**

The following equation, known as the "isotope balance" or "isotope dilution" equation can be used to obtain the desired $^{15}$N enrichment when for example N fertilizers labelled with different enrichments are mixed:

$$ x(a_1) + y(a_2) = (x + y) \tilde{a} $$

(1)
Where \( x \) = quantity of material with \( ^{15}\text{N} \) abundance \( a_1 \) (of higher \( ^{15}\text{N} \) enrichment) and \( y \) = quantity of material with \( ^{15}\text{N} \) abundance \( a_2 \) (of lower enrichment).

\( \bar{a} \) = average or desired \% \( ^{15}\text{N} \) abundance in the final mixture of \( x+y \).

If the added diluting material is an unlabelled (ordinary) fertilizer, then

\[ a_2 = a_0 = 0.37\% \, ^{15}\text{N} \text{ abundance} = 0\% \, ^{15}\text{N} \text{ at. excess} \]

Equation (1) then becomes

\[ \frac{x \, (a'_1)}{x + y} = \bar{a}' \quad (2) \]

Where \( a'_1 = \% \, ^{15}\text{N} \text{ at. excess of material of higher } ^{15}\text{N} \text{ enrichment} \)

\( \bar{a}' = \% \, ^{15}\text{N} \text{ at. excess desired in the final mixture}. \)

In earlier exercises, it was shown that compounds, which differ substantially in their \( ^{15}\text{N} \) enrichment have different molecular masses or molecular weights. However, these differences tend to be small with materials whose enrichments are similar. In this case, \( M_1 = M_2 \) (where \( M_1 \) and \( M_2 \) represent the molecular masses of two compounds) and the amounts of \( x \) and \( y \) can be expressed as \( m_1 \) and \( m_2 \) in mass units (grams). Thus equation (2) can be rewritten as follows:

\[ m_1 = \frac{(m_1 + m_2) \, \bar{a}'}{a'_1} \quad (3) \]

When the enrichments are very different (\( M_1 \neq M_2 \)), the amounts of \( x \) and \( y \) must be expressed as \( n_1 \) and \( n_2 \), i.e. in number of moles of each compound. In this instance, equation (2) becomes:

\[ \bar{a}' = \frac{n_1 \, (a'_1)}{n_1 + n_2} \quad (4) \]

or

\[ \bar{a}' = \frac{m_1 \, M_2 (a'_1)}{m_1 M_2 + m_2 M_1} \quad (5) \]

and

\[ m_1 = \frac{(m_1 + m_2) \, M_1 \bar{a}'}{M_2 a'_1 + (M_1 - M_2) \, a'} \quad (6) \]
Exercise 6:

Preparation of solutions of required $^{15}$N content. From a stock of 2.4 at. % $^{15}$N abundance $(\text{NH}_4)_2\text{SO}_4$ and ordinary $(\text{NH}_4)_2\text{SO}_4$, make up 10 liter solution containing 140 g $(\text{NH}_4)_2\text{SO}_4$ with 1.2 at. % $^{15}$N abundance.

Calculations:

Assuming that $M_1 = M_2$ (i.e. there is no appreciable difference in enrichments).

Thus, $m_1 + m_2 = 140$ g $(\text{NH}_4)_2\text{SO}_4$

2.4 % $^{15}$N abundance, 2.4 - 0.37 = 2.03 % $^{15}$N at. excess = a

1.2 % $^{15}$N abundance desired = 1.2 - 0.37 = 0.83 % $^{15}$N at. excess = $a'$

Then,

$m_1 = 140 \times \frac{0.83}{2.03} = 57.2$ g $(\text{NH}_4)_2\text{SO}_4$ of 2.4 at. % $^{15}$N abundance.

and

$m_2 = 140 - 57.2 = 82.8$ g ordinary $(\text{NH}_4)_2\text{SO}_4$

Exercise 7:

Calculation of $^{15}$N fertilizer requirements for a field experiment. In a field experiment on nitrogen fixation (see Hardarson and Danso, this volume), there are a total of 24 plots. A legume crop is to be grown on 18 of these plots, while the remaining 6 plots are to be planted with the reference or non fixing crop. The legume will receive 20 kg N/ha of 5 at. % $^{15}$N excess ammonium sulphate, while the reference crop receives 100 kg N/ha of 1 at. % $^{15}$N excess ammonium sulphate. The plot sizes are 3.6 m$^2$ for the legume, and 1.8 m$^2$ for the reference crop. The available fertilizer stock consists of ammonium sulphate with 50 at. % $^{15}$N abundance and unlabelled ammonium sulphate. How would you prepare these solutions?
Legume (Faba bean)  
5 at. % $^{15}$N excess 

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N rate</td>
<td>20 kg/ha</td>
</tr>
<tr>
<td>g N/m²</td>
<td>2</td>
</tr>
<tr>
<td>plot size</td>
<td>3.6 m²</td>
</tr>
<tr>
<td>g N/plot</td>
<td>7.2</td>
</tr>
<tr>
<td>$g(NH_4)_2SO_4$</td>
<td>33.9</td>
</tr>
<tr>
<td>number of plots</td>
<td>18</td>
</tr>
<tr>
<td>** aliquot of solution**</td>
<td>200 ml**</td>
</tr>
<tr>
<td>per plot</td>
<td></td>
</tr>
<tr>
<td>total volume</td>
<td>18x200 = 3600 ml</td>
</tr>
<tr>
<td>for fertilizer + 200 ml</td>
<td></td>
</tr>
<tr>
<td>standard + spillage</td>
<td>3800 ml</td>
</tr>
</tbody>
</table>

*** amount of 664 g fertilizer for the experiment

\[ X = 33.87 \text{ g } (NH_4)_2SO_4 \]

\[ Y = 84.89 \text{ g } (NH_4)_2SO_4 \]

** These aliquots have to be diluted to e.g. 4 or 8 l, respectively, for homogeneous application.

Standard crop (Barley)  
1 at. % $^{15}$N excess 

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N rate</td>
<td>100 kg/ha</td>
</tr>
<tr>
<td>g N/m²</td>
<td>10</td>
</tr>
<tr>
<td>plot size</td>
<td>1.8 m²</td>
</tr>
<tr>
<td>g N/plot</td>
<td>18</td>
</tr>
<tr>
<td>$g(NH_4)_2SO_4$</td>
<td>84.9</td>
</tr>
<tr>
<td>number of plots</td>
<td>6</td>
</tr>
<tr>
<td>** aliquot of solution**</td>
<td>400 ml**</td>
</tr>
<tr>
<td>per plot</td>
<td></td>
</tr>
<tr>
<td>total volume</td>
<td>6x400 = 2400 ml</td>
</tr>
<tr>
<td>for fertilizer + 200 ml</td>
<td></td>
</tr>
<tr>
<td>standard + spillage</td>
<td>2600 ml</td>
</tr>
</tbody>
</table>

*** amount of 552 g fertilizer for the experiment

\[ X = \frac{33.9 \times 3800}{200} = 644 \text{ g} \]

\[ Y = \frac{84.9 \times 2600}{400} = 552 \text{ g} \]
Dilution of 50 at. % $^{15}$N abundance (M₁=133 g/mol) ammonium sulphate with ordinary (natural abundance) ammonium sulphate (M₂=132 g/mol) to prepare:

a) 644 g (NH$_4$)$_2$SO$_4$ of $\bar{a}' = 5$ at. % $^{15}$N excess

Using equation (6) for $M_1 \neq M_2$

$$m_1 = \frac{644 \times 133 \times 5}{132 \times 49.63 + (133-132)\times 5}$$

$$m_1 = 65.32 \text{ g of 50 % (NH}_4)_2\text{SO}_4$$

$$m_2 = 644 - 65.32 = 578.68 \text{ g ordinary (NH}_4)_2\text{SO}_4$$

Using equation (3) for $M_1 = \text{H}_2$

$$m_1 = \frac{(m_1 + m_2) \bar{a}'}{a'} = \frac{644.5}{49.63}$$

$$m_1 = 64.88 \text{ g 50 % (NH}_4)_2\text{SO}_4$$

$$m_2 = 644 - 64.88 = 579.12 \text{ g ord. (NH}_4)_2\text{SO}_4$$

The error is very small in both cases for practical work. In this case 65 g of 50 at. % $^{15}$N ab. (NH$_4$)$_2$SO$_4$ and 579 g ord. (NH$_4$)$_2$SO$_4$ is dissolved in water to make a total volume of 3800 ml solution.

b) 552 g (NH$_4$)$_2$SO$_4$ of $\bar{a}' = 1$ at. % $^{15}$N excess

The previous calculation shows that the error is very small when equation (3) is used therefore,

$$m_1 = \frac{552 \times 1}{49.63} = 11.1 \text{ g of 50 % (NH}_4)_2\text{SO}_4$$

$$m_2 = 552 - 11.1 = 540.9 \text{ g ordinary (NH}_4)_2\text{SO}_4$$

The 1 at. % $^{15}$N exc. labelled ammonium sulphate solution would be prepared by dissolving 11 g of 50 at. % $^{15}$N abundance and 541 g of ordinary ammonium sulphate in water, making up a total volume of 2600 ml solution.
Many of the concepts which have been presented on stable isotopes are equally useful in agricultural experiments with radioisotopes. In fact, concepts such as the A-value and isotope dilution (see Zapata, this volume) were developed with radioisotopes. Experiments with radioisotopes are normally less expensive to conduct since their analysis requires less sophisticated equipment than stable isotopes. The additional factor of radiation protection may prevent scientists without adequate facilities from conducting research with radioisotopes. Acceptable safety standards are outlined in other IAEA publication (IAEA, 1973 and 1976).

Units

The activity of a radioactive source or radionuclide sample is, by definition, its strength or intensity. In other words, it is the number of nuclei decaying per unit of time. The SI unit of radioactivity is becquerel (symbol Bq). 1 Bq is one disintegration per second (dps). The unit curie (Ci) was used previously instead of becquerel (Table 6).

<table>
<thead>
<tr>
<th>Radioactivity units</th>
<th>Activity (Bq)</th>
<th>Disintegration Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curie</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Ci</td>
<td>$3.7 \times 10^{10}$ Bq</td>
<td>$3.7 \times 10^{10}$ dps</td>
</tr>
<tr>
<td>1 mCi</td>
<td>$3.7 \times 10^{7}$ Bq</td>
<td>$3.7 \times 10^{7}$ dps</td>
</tr>
<tr>
<td>1 μCi</td>
<td>$3.7 \times 10^{4}$ Bq</td>
<td>$3.7 \times 10^{4}$ dps</td>
</tr>
<tr>
<td>1 nCi</td>
<td>$3.7 \times 10^{2}$ Bq</td>
<td>$3.7 \times 10^{2}$ dps</td>
</tr>
<tr>
<td>1 pCi</td>
<td>$3.7 \times 10^{-2}$ Bq</td>
<td>$3.7 \times 10^{-2}$ dps</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Radioactivity units (Ci equivalent)</th>
<th>Activity (Bq)</th>
<th>Disintegration Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>27,027 mCi</td>
<td>1 GBq</td>
<td>$1 \times 10^{9}$ dps</td>
</tr>
<tr>
<td>27,027 μCi</td>
<td>1 MBq</td>
<td>$1 \times 10^{6}$ dps</td>
</tr>
<tr>
<td>27,027 nCi</td>
<td>1 KBq</td>
<td>$1 \times 10^{3}$ dps</td>
</tr>
</tbody>
</table>
**Decay law, half-life**

All radioisotopes decay exponentially with time. It is through nuclear disintegration that ionizing radiation is emitted in the form of alpha (α) or beta (β) particles or gamma (γ) rays.

Thus, each radioisotope has the following characteristics:

1) Constant rate of decay (λ) and corresponding half-life \( t_{1/2} \).
2) Decay scheme and type of ionizing radiation (\( α, β, γ \)).
3) Energy pattern of the particles or rays.

These concepts are explained in more detail in IAEA (1976).

Rates of radionuclide decay are usually expressed in terms of half-life \( t_{1/2} \). The half-life of a radioisotope is defined as the time required for half of the initial radioactive atoms to undergo decay or in other words the radionuclide "lose half of their initial radioactivity". This is illustrated in Fig. 1.

![32P decay, \( t_{1/2} = 14.3 \) d](image)

**FIG. 1.** Linear graph of the \( ^{32}\text{P} \) decay.
The radioactivity of $^{32}\text{P}$ is reduced by half after every interval of 14.3 days, thus $t_{1/2}$ of $^{32}\text{P} = 14.3\text{ d}$. Since the radionuclide decay is a logarithmic relation, straight line is obtained if plotted on log-paper.

Radionuclide decay may best be defined in mathematical terms. The number (dN) of atoms disintegrating in a given time (dt), is proportional to the number (N) of radioactive atoms present. This relationship may be written as:

$$\frac{dN}{dt} = -\lambda N$$  \hspace{1cm} (7)

where $\lambda$ is the decay constant. If the differential equation (7) is integrated between the limits of $N_o$ and $N$, and $t_o$ and $t$, where $N_o$ represent the number of radioactive atoms at zero time $t_o$, then the following equation results:

$$\int_{N_o}^{N} \frac{dN}{N} = -\lambda \int_{t_o}^{t} dt$$

and

$$\ln \frac{N}{N_o} = -\lambda t$$

$$\ln N = \ln N_o - \lambda t$$  \hspace{1cm} (8)

where $\ln$ is the natural logarithm or

$$\log N = \log N_o - \left\{\frac{\lambda}{2.3} x t\right\}$$

where $\log$ is the common logarithm (base 10).

Since the activity (A) of a substance is actually its decay intensity and this is proportional to the number of radioactive atoms, equation (8) may be written as:

$$\ln A = \ln A_o - \lambda t$$  \hspace{1cm} (9)

To use equations (8) or (9) the decay constant ($\lambda$) must be known. After an interval of time corresponding to the half-life ($t_{1/2}$), half of the original activity remains, we may write equation (9) as:

$$\ln 1/2 = \ln 1 - \lambda t_{1/2}$$
or
\[ \ln 2 = \lambda t_{1/2} \]
or
\[ 0.693 = \lambda t_{1/2} \]

therefore,
\[ \lambda = \frac{0.693}{t_{1/2}} \] (10)

The following example illustrates how to use equation (9) to calculate the decay of a $^{32}$P-sample.

Exercise 8:

A sample contains 37 K Bq ($= 1\mu$Ci = $3.7 \times 10^4$ dps) of $^{32}$P at $t_0$. What will be its activity after 30 days?

The half-life ($t_{1/2}$) of $^{32}$P is 14.3 days

\[
\ln A = \ln 3.7 \times 10^4 - (\lambda \times 30) \quad \lambda \text{ see equation (10)}
\]

\[
\ln A = \ln 3.7 \times 10^4 - \left\{ \frac{0.693}{14.3 \text{ d}} \times 30 \text{ d} \right\}
\]

Observe, that the units for half-life and decay time have to be the same, in this example days (d)!

\[
\ln A = 10.5186 - 1.4538
\]
\[
\ln A = 9.0648
\]
\[
A = 8646 \text{ dps}
\]

In 30 days the activity decreased from 37.000 dps to 8646 dps or from 37 K Bq ($1 \mu$Ci) to 8.6 K Bq ($0.23 \mu$Ci).

The decay of this sample can be also roughly estimated with the following relationship:

after one half-life, \[ \frac{A}{2} \] of the initial activity remains, \[ N = \frac{N_0}{2} \]

after two " " \[ \frac{A}{2^2} " " " " " " N = \frac{N_0}{2^2} \]
Thus in the forementioned example,

\[
\frac{30 \text{ days}}{14.3 \text{ days}} = 2.1 \text{ half-lives} = n \quad \text{or approx. } n = 2
\]

\[
\frac{A}{2^2} = \frac{37 \text{ K Bq}}{4} = \frac{37 \text{ K Bq}}{4} = 9.25 \text{ K Bq.}
\]

or \[
\frac{37,000}{4} = 9250 \text{ dps}
\]

**Specific activity**

A radioisotope is generally accompanied by stable isotope of the same element. Specific activity is the amount of radioactivity per unit weight (or volume) of total element present, including both active and stable isotopes. Various expressions may be used, such as Bq/g, μCi/g or dpm/mg, dps/mg etc.

**Exercise 9:**

Two plant samples were analysed for both the radioisotope \(^{32}\text{P}\) and total P. An acid extract was prepared and in each case an 10 ml aliquot was used for Cerenkov counting. Sample (a) gave 1000 cpm, the counting efficiency was 40%; sample (b) gave 1000 cpm and the counting efficiency was 20%. The differences in efficiency was due to different colour of the extracts. The total P-content in the 10 ml aliquots was in both samples 10 mg.

Is the specific activity of these two samples the same?

If the counts per minute (cpm) are used for the calculation, the answer would be yes, which is **wrong**!

Using correctly the dpm of the samples (which means correction for the different counting efficiencies) the following result will be obtained:

Sample (a) has 1000 cpm which is only 40% of the dpm value, in other words the sample has an activity of 2500 dpm.

**Specific activity of sample (a) = \(\frac{2500 \text{ dpm}}{10 \text{ mg P}} = 250 \text{ dpm/mg}\)**
Sample (b) has 1000 cpm which is only 20% of the dpm value, in other words the sample has an activity of 5000 dpm.

\[
\text{Specific activity of sample (b)} = \frac{5000 \text{ dpm}}{10 \text{ mg } P} = 500 \text{ dpm/mg P}
\]

**Estimate of radioisotope requirements for soil-plant experiments:**

In experiments with radioisotopes, it is necessary to make reasonable estimates of the specific activity and quantity of labelled fertilizer. When calculating the total isotope requirements, the specific activity is the first parameter to be determined in order to have enough radioactivity at the sampling time and ensure a satisfactory counting rate in the harvested plant material. A count rate of 30-35 cps or about 2000 cpm is considered optimum for most soil/plant experiments. Three major factors will determine the specific activity of the labelled fertilizer to be used: (a) the efficiency of the counting system, (b) the extent of chemical and biological dilution, and (c) the length of the growing period in relation to the decay of radioactivity and some other specific properties of the isotope. Specific activities of ~20 M Bq (0.5 mCi) \(^{32}\)P/g P or 2M Bq (0.05 mCi) \(^{33}\)P/g P are frequently used in field studies.

In order to estimate the quantity of labelled fertilizer needed, the following agronomic specifications must be known: the fertilizer source and its nutrient content, the fertilizer rate, the plot area and total number of plots in the experiment. The calculated amount of labelled fertilizer should be increased by 30% to have material for counting standards, to allow for spillage, and to make allowance for time to prepare the labelled fertilizer.

**Exercise 10:**

Estimate radioisotope requirement for a maize experiment given the following basic data:

Growth period (seeding to harvest) = 10 weeks (70 days). 2g dry sample contains 0.25% P and % P derived from fertilizer (%Pdff) = 10%. Assume a counting efficiency of 40% (Cerenkov counting).
Agronomic specifications:

Phosphorus source: Ordinary superphosphate (OSP), 8.7% P
P rate: \(40 \text{ kg P/ha} = 4 \text{ g/m}^2\)
Plot size: \(3.00 \times 1.50 \text{ m} = 4.5 \text{ m}^2\)
Total number of plots: \(= 18 \) (3 treatments x 6 replications)

Solution:

- Starting from a desired sample count rate of 2000 cpm/2 g plant material and considering the counting efficiency \(\frac{100}{40} = 2.5\), the final activity \((N)\) should be 5000 dpm/2 g plant material.

- The experiment will last for 70 days, that means about 5 half-lives for \(^{32}\text{P}\); then the initial activity \((N_0)\) will be:

\[
N_0 = N \times 2^N
\]

\[
N_0 = 5000 \times 2^5
\]

\[
N_0 = 5000 \times 32 = 160,000 \text{ dpm} = 16 \times 10^4 \text{ dpm}
\]

\[
N = \frac{16 \times 10^4 \text{ dpm}}{60} = 2.6 \times 10^3 \text{ dps} = 2.6 \text{ K Bq/2g plant material}
\]

- Correction for chemical and biological dilution:

Amount of P in the sample coming from fertilizer
\[
= 2 \times \frac{0.25}{100} \times \frac{10}{100} = \frac{5}{10^4} \text{ or } 5 \times 10^{-4} \text{ g P}
\]

This amount would then be equivalent to a specific activity of:

\[
\frac{2.6 \text{ K Bq}}{5 \times 10^{-4} \text{ g P}} = 5.2 \text{ M Bq}^{32}\text{P per g P}
\]

Therefore the \(^{32}\text{P}\)-labelled fertilizer should have a minimum specific activity of 5.5 M Bq/g P at the beginning of the experiment.

- Calculation of the amount of \(^{32}\text{P}\)-labelled fertilizer:

Total area to be labelled = 18 plots x 4.5 m\(^2\)/plot = 81 m\(^2\)
Total amount of P
\[
= 81 \text{ m}^2 \times 4 \text{ g P/m}^2 = 324 \text{ g P}
\]

Estimated amount of \(^{32}\text{P}\)-labelled fertilizer
\[
= 324 \times \frac{100}{8.7} = 3724 \text{ g OSP}
\]

Net amount to be ordered is 4 kg, considering an extra amount of 10%.
If it is assumed that one additional half-life (14 days) is the time necessary for customs clearance, delivery and preparation of field experiment, the initial specific activity should be 10 M Bq $^{32}$P/g P.

The total activity for the experiment should therefore be:

$$324 \text{ g P} \times 10 \text{ M Bq/g P} = 3240 \text{ M Bq} = 3.2 \text{ G Bq}$$

Choosing an appropriate isotope of phosphorus

Phosphorus has two radioisotopes which are convenient for agricultural research. The one most frequently used is $^{32}$P with a half-life of 14.3 days, although $^{33}$P, with a half-life of 25.3 days has certain advantages. Both isotopes emit beta particles, but $^{32}$P has a beta particle with higher energy (1.7 MeV) than $^{33}$P (0.25 MeV). $^{32}$P can thus be counted with GM systems or by Cerenkov techniques, while a liquid scintillation counter is needed to obtain satisfactory efficiency with $^{33}$P. The weaker radiation of $^{33}$P provides higher resolution on autoradiographs. However a major factor in choosing the most suitable isotope for a field experiment is the isotope cost, which is higher for $^{33}$P than for $^{32}$P.

In the previous exercise 10 illustrating a field study conducted for 70 days, where $^{32}$P would decay through 5 half-lives to $1/2^5 = 1/32$ of its initial activity, $^{33}$P would only decay to $1/2^3$ or $1/8$ of its activity. If we assume similar counting efficiency, etc., only 1/4 of the activity would be required, if the study was conducted with $^{33}$P.

Since the cost of $^{33}$P is more than 4 times higher than $^{32}$P the latter will still be more economical. Only for experiments lasting for more than 120 days it is preferable to use $^{33}$P labelled fertilizer, because a very high specific activity of $^{32}$P would be required (200 M Bq $^{32}$P/g P). Furthermore high radiation safety precautions would be required for handling the fertilizer and it is possible that the radiation could affect the plant growth.
REFERENCES


FIELD EXPERIMENTATION IN ISOTOPE-AIDED STUDIES

F. ZAPATA
Soil Science Unit,
FAO/IAEA Programme,
Agency’s Laboratory,
International Atomic Energy Agency,
Seibersdorf

INTRODUCTION

Isotopic-aided studies involve the application of isotopically labelled fertilizer as tracers for the quantitative and precise determination of the fate of specific nutrient elements in the soil/plant system. The planning of isotopic-aided studies requires a different approach from that followed in the design of normal fertilizer trials because of the cost and supply of isotopically labelled materials, the use of highly specialized equipment and the need for skillful trained staff in the use of isotope techniques both in the field/greenhouse and the laboratory.

This section is intended to highlight the main points to be considered while applying those techniques in the field or greenhouse.

It has been well established that nuclear techniques are a powerful tool in agricultural research. One should take advantage of the use of such techniques if the following criteria are met:

1) The isotope method is the only way to solve a particular question or to obtain a specific piece of information.

2) There are other methods available for such a purpose but the nuclear method provides a direct and quick means to obtain the needed information resulting in higher economic return.

Since nuclear techniques are normally complementary to conventional or classical techniques in agricultural experimentation, the research team should ideally consist of scientists not only trained in the use of nuclear techniques but also specialized in the research topic under study. Experience in developing countries through IAEA technical co-operation projects (TCP) has demonstrated that the best results are obtained when co-operation agreements are established between specialized groups of Agricultural Research Institutes/Universities and the nuclear experts from Atomic Energy Commissions.
There is a logical sequence in the application of nuclear techniques in agricultural research. The main steps to be considered are the following:

1) Definition of the main research topic to be studied.
2) Compilation of all available background information on this topic.
3) Elaboration of a research work plan, including the identification of the specific topics or particular questions to be solved using nuclear techniques.

During the planning of the isotopic-aided experiments the objectives will be defined in relation to the particular questions being asked.

A general rule: Design simple experiments with concrete and well-defined objectives. Experience from past Co-ordinated Research Programmes (CRP) showed that the required information can be better obtained through a series of simple experiments than through a single complicated experiment.

THE RESEARCH PROJECT CYCLE

The detailed planning of an isotopic-aided experiment should always involve the writing of an experimental guideline which can be improved after review by the members of the research team. The final version should be prepared by the chief investigator responsible for the research work.

The following list includes the points normally considered in the experimental guidelines.

1. Introduction

The author should provide in a clear and concise way the basic information on the research topic being studied and the role of the isotope technique(s) in the study.

2. Objectives

Each experiment should only have one or two clearly stated objectives.

3. Treatments and experimental design

The experimental treatments should be defined in direct relation to the objectives.
The number of treatments and replications per treatment are a function of each experiment. The decision on the total number of experimental units should be based on technical and economic considerations. Do not plan experiments based only on economic considerations, i.e.: availability of isotopically-labelled fertilizer.

Basic principles of statistical analyses should be considered in selecting the appropriate experimental design. Past experimental plans of CRP on fertilizer use efficiency included a core of 4 to 6 treatments (common to all researchers participating in the CRP) and 2 to 4 optional treatments (left up to the decision of each researcher). Randomized block arrangement with 4 to 6 replications per treatment was the most commonly used statistical design. Also split-plot design was often used in several fertilizer uptake experiments.

The preparation of diagrams illustrating the plot and field layout is essential for the research team during field observations as well as for the field staff during sowing, maintenance and harvesting operations. The establishment of the plot layout depends on the plant species and the cropping system. In addition a clear distinction should be made between yield and isotope plots. The isotope plots are usually microplots covering the smallest possible area required to obtain a representative sample for a good estimate of the isotopic parameter. The isotopically labelled fertilizer is applied to these small plots. Microplot size may vary from about 1m² (pastures, small grain cereals) to about 10m² (widely-spaced row crops). In our experience a representative plant sample would consist of at least 20 plants to be obtained from the harvested area of the microplots. The yield plots cover a relatively larger (10-15 m²) area, their objective being to obtain a precise estimate of biomass production, economic crop (grain, tuber, etc.) yield and total nutrient uptake. In these plots, it is also possible to get any additional information; e.g.: crop growth measurements, physiological parameters, water measurements, nodule observations in case of nodulated plants, etc. The experimental field layout or the spatial arrangement of plots (yield and isotope) depends to a large extent on the objective of the experiment.

4. Isotopically labelled fertilizer

In isotope-aided field experiments on fertilizer practices only one single fertilizer rate of application is needed, namely that one normally recommended for optimum yield. In case of split application, a single rate of application
for instance 100 kg N/ha can be divided into two (50 and 50 kg N/ha), three (33, 33 and 33 kg N/ha) or four (25, 25, 25 and 25 kg N/ha) equal fractions to be applied at different times throughout the growth cycle.

In principle a control or check treatment (without fertilizer application) is not needed in isotope-aided field experiments, because fertilizer nutrient uptake is measured directly using labelled fertilizer.

Stable isotopes: $^{15}$N application

The rate of $^{15}$N application must be sufficient to be traced in the whole or part of the soil/plant system, at the time of harvest. The amount of $^{15}$N applied depends on both the rate of N application and the enrichment (% $^{15}$N atom excess) of the $^{15}$N-labelled fertilizer used. This amount is determined by several factors such as purpose of the study, type of crops, duration of the experiment, and isotopic ratio determination equipment. Based on past experience, as a rule of thumb, 1 kg $^{15}$N/ha (if nitrogen isotopic ratios are determined by mass spectrometry) or 2 kg $^{15}$N/ha (if optical emission spectrometry is used) are required for annual crops in plant fertilizer N recovery studies. In case of fertilizer N balance (plant and soil fertilizer N recovery) studies, about 10 kg $^{15}$N/ha or 20 kg $^{15}$N/ha would be required depending on the measurement equipment available. In special studies such as downward movement of $^{15}$N-labelled fertilizer, leaching/runoff losses, $^{15}$N incorporation in soil N fractions, ammonia volatilization, etc. at least 50 kg $^{15}$N/ha would be required. In all cases, the amount of $^{15}$N to be applied for the experiments must be tested by the researchers themselves. After some years of experience they must be able to select the adequate amounts of $^{15}$N to be used in their own experiments.

Radioisotopes: $^{32}$P application

The amount of $^{32}$P to be applied must be just high enough for an accurate determination of the radioisotope (counting rate) with the available detection equipment in the harvested plant material at the end of the experiment. A counting rate of 30-35 cps or about 2000 cpm is considered optimum for most soil-plant experiments. Three major factors will determine the specific activity of the labelled fertilizer to be used: (a) the efficiency of the counting system, (b) the extent of chemical and biological dilution, and (c) the length of the growing period in relation to the decay of radioactivity and some other specific properties of the isotope. Specific activities of 20 MBq
(0.5 mCi) $^{32}\text{P}$/g P or 2 MBq (0.05 mCi) $^{33}\text{P}$/g P are frequently used in field studies.

The planning of experiments with radioisotopes requires the preparation of a detailed schedule of all activities and the conduct of dummy trials. Please consider carefully safety regulations before initiating experiment using radioisotopes.

The total amount of activity (some hundreds of mCi) required for a field experiment depends on the rate of P and specific activity of the P fertilizer applied as well as the size of experiment.

The exercise No. 10 (see Axmann and Zapata, this volume) illustrates the calculation utilized to estimate the required amount of P fertilizer, its specific activity and the total amount of activity.

Calculations of isotopically labelled fertilizer

Following the guidelines mentioned above, the total fertilizer requirements for an experiment are calculated as follows:

i) The amount required per row (one lot) for a given treatment.

ii) The amount required per replication or plot, (X lots, where $X =$ number of rows) for a given treatment.

iii) The amount required per experiment (Y lots, where $Y =$ number of rows x number of replications) for a given treatment.

iv) Total fertilizer requirements for all treatments of the experiment.

v) Finally it is possible to make a cost estimate of the labelled fertilizer requirements, based on recent bid quotations from commercial suppliers.

Fertilizer application

In order to draw direct conclusions about fertilizer nutrient (N or P) uptake and utilization, the isotopically labelled fertilizers should be chemically (carrier) and physically (form) identical to the commercial fertilizers.
Most fertilizers are applied in solid, dry form. The fertilizer's lots, are weighed out and applied as homogenously as possible, on a row basis. Chemical source, physical form (crystal, powder, prilled, granulated, etc.) time and methods of application are factors left up to the decision of the research team for the development of fertilizer management practices.

5. Harvest and preparation of plant samples

It should always be kept in mind that the primary objective of the microplots (in isotopic-aided studies) is to obtain a precise estimate of the isotopic parameter. This determination will serve as a basis for the quantitative comparison of fertilizer treatments. Therefore the time for harvesting should be set for collecting the biomass produced and the total amount of nutrient (N or P) taken up during the length of the experiment. Sometimes several harvests are made throughout the duration of the experiment to study time course of nutrient uptake. The final harvest time should not be later than physiological maturity to avoid leaf shedding, seed shattering, etc. and other physiological phenomena of advanced maturity, which could greatly increase the overall experimental error. If exact yield (grain, tuber) data are required, this measurement should be done on the yield plots at full maturity.

The harvested area of the isotope plots (microplots) normally comprises the middle of the labelled plot (middle length of 2-3 central rows) leaving the remaining plot area (extreme ends of the central rows and outer rows) as border.

Harvesting procedure consists of gathering all above-ground plant material (and exceptionally roots) in the harvested area of an isotope plot and treating it as a sample. Avoid contamination of plant samples with treated soil. Roots have to be washed very carefully if harvested.

The harvested plant samples, duly identified are transported from the field to a sample's preparation area (covered area with facilities for weighing, chopping and drying the plant material). All the sample preparation procedures are made on the plant samples carefully organized per treatment and replication. The ultimate goal being to obtain a representative sample for isotope analyses.
SAMPLE PREPARATION TECHNIQUES OF BIOLOGICAL MATERIAL FOR ISOTOPE ANALYSIS

H. AXMANN, A. SEBASTIANELLI, J.L. ARRILLAGA
Soil Science Unit, FAO/IAEA Programme, Agency's Laboratory, International Atomic Energy Agency, Seibersdorf

SAMPLING AND SUBSAMPLING PROCEDURES

Sample preparation is an essential step in all isotope-aided experiments but often it is not given enough attention. The methods of sample preparation are very important to obtain reliable and precise analytical data and for further interpretation of results. The size of a sample required for chemical analysis is usually very small (10mg-1500mg). On the other hand the amount of harvested plant material from plots in a field experiment is often bulky (several kilograms) and the entire sample is too large for processing. In addition, while approaching maturity many crops show not only differences in physical consistency but also a non-uniformity in $^{15}$N content among plant parts, requiring a plant fractionation or separation into plants (vegetative and reproductive), e.g: shoots and spikes, in case of small grain cereals, shoots and pods in case of grain legumes and tops and roots or beets (including crown) in case of sugar beet, etc. In any case the ultimate goal of these procedures is to obtain representative subsample harvested from greenhouse or field experiments for chemical analysis.

A diagramatic representation of the steps, which have to be followed in the sampling and subsampling procedures is shown in Fig. 1. Before harvesting an isotopic-aided experiment the method of sampling has to be selected. It should be based on the type of information required in relation to the objectives of the research and the availability of resources (staff, sample preparation equipment, analytical facilities, chemicals and supplies, etc.).

In order to obtain reliable and precise analytical data, the following precautions are required:

1. A careful organization of samples is essential to avoid cross-contamination during chopping and grinding procedures. This means that one should always start with samples of lowest $^{15}$N enrichment followed by increasing enrichments. In this way cross contamination problems are kept very low, even during routine work.
2. A proper identification of field samples coming from the harvested plots and the prepared subsamples which are sent for chemical and isotopic analysis is essential. All these information should be entered in a field record book. It is customary to prepare working tables, to record such information. If the samples will be analysed elsewhere, this complete information should be sent to the laboratory rendering the analytical services together with the subsamples and fertilizer standard(s) used in the experiment. Past experience from programmes of the Joint FAO/IAEA Programme indicated that many mistakes can arise if these recommendations are neglected.

The following steps are shown in Fig. 1:

FIG. 1. Flow diagram of sampling and sub-sampling of labelled samples from a field experiment.
First the total fresh weight (TFW) of the sample (harvested plant material) is recorded when the entire plant sample is to be processed. Sometimes, however, according to the objectives of the research, a plant sample separation or fractionation, will be required. In this case the fresh weight of the plant parts is recorded separately. The fresh plant material is then cut or chopped into 1-3 cm pieces with adequate machinery (chopping machine) or tools (knives, scissors). The chopped material is then mixed thoroughly by placing it on a large sheet of paper or plastic foil, rolling the sample back and forth in all directions. The well mixed sample is piled in the centre of the sheet. Quarter the sample and save the two opposite quarters as illustrated in Fig. 2. Repeat the process with the saved subsample until the sample size has decreased to 200-300 g fresh weight. The sample is placed in a paper bag. Record the exact fresh weight of the subsample (SFW) and dry in an oven (18-24 hours) at 70°C until constant weight is reached.

![FIG. 2. Quartering.](image)

It is very important that the weights of the total fresh sample (TFW) and the subsample (SFW) are taken within a short period. In other words, the plant material should not lose any water between these two weighings. If this happens, the determined moisture content of the sample will be wrong, which leads to wrong estimates of dry matter yield. When different organs are analysed separately, it is even more important to get correct dry matter data.

The weight of the dry subsample (SDW) is recorded and the entire subsample is finely ground to pass a 1 mm sieve.
The following table serves an example for recording all necessary data:

**Table 1: Sampling and sub-sampling**

<table>
<thead>
<tr>
<th>Sample coding</th>
<th>T.F.W.</th>
<th>Sub-sample</th>
<th>T.D.W.</th>
<th>Dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatm.</td>
<td>Plant</td>
<td>IN g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. Repl.</td>
<td>Part (From area harvested)</td>
<td>S.F.W.</td>
<td>S.D.W.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>in g</td>
<td>in g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 l</td>
<td>Pods</td>
<td>850</td>
<td>270</td>
<td>37.8</td>
</tr>
<tr>
<td>2 l</td>
<td>Shoots</td>
<td>2980</td>
<td>247</td>
<td>43.8</td>
</tr>
</tbody>
</table>

TPW: total fresh weight  
SFW: sub-sample fresh weight  
SDW: sub-sample dry weight  
TDW: total dry weight

After the samples are finely ground it is not necessary to analyse the different parts of the plant separately. Utilizing the ratios of the total dry matter weights one can again mix sample (each treatment and replication) in the ratio of TDW of plant parts, e.g. in the above mentioned example the TDW of shoots is 528 g, and the pods are 119 g. The ratio is 4.44:1, therefore we may mix 8.88 g shoots material + 2.00 g pods material. Make sure that these two samples are well mixed and analyse this composite sample.

**CHEMICAL TREATMENT OF $^{15}$N-LABELLED BIOLOGICAL MATERIAL FOR TOTAL NITROGEN AND $^{15}$N ANALYSES**

Measurements of the $^{15}$N/$^{14}$N isotopic ratio in biological material is carried out either by mass- or emission-spectrometer. In both cases only nitrogen gas can be used for the analyses. Therefore the nitrogen compounds in a sample have to be converted into nitrogen gas. In principle there are two methods available; the Kjeldahl-Rittenberg oxidation and the Dumas-dry combustion (Fig. 3).
Kjeldahl-Rittenberg Oxidation

The reactions of the Kjeldahl-Rittenberg oxidation take place in solution. This method includes the following steps:

1) Digestion:

The digestion is performed to convert organic and inorganic N into ammonium salts. The digestion of the sample is accomplished in concentrated sulfuric acid with additives such as hydrogen peroxide or suitable catalysts in order to promote oxidation of organic matter. The conversion to ammonium salts as shown by the following general reaction:

$$\text{Sample} + H_2SO_4 \text{ conc.} \rightarrow (NH_4)_2SO_4 + CO_2$$

Although the Kjeldahl method has been used for more than 100 years and several hundred papers have been published on the technique, there is still no universally accepted digestion method. The best compromise seems to be to adapt the digestion technique to the nature of the sample material.
The following procedure for digestion of plant material can serve as an example for semi-micro Kjeldahl digestion:

(i) For samples without nitrate and nitrites

a) Place a weighed sample (300-1500 mg) into a dry 100 ml Kjeldahl flask. The weight will depend on the nitrogen content of the material, it should include 3-20 mg nitrogen (Table 2).

Table 2: Mean total nitrogen content (%) of some materials

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SOILS</strong></td>
<td>0.05 - 0.25</td>
</tr>
<tr>
<td><strong>PLANTS</strong></td>
<td></td>
</tr>
<tr>
<td>grass, clover, alfalfa (fresh)</td>
<td>0.5</td>
</tr>
<tr>
<td>grass, clover (dry matter)</td>
<td>1.4 - 2.7</td>
</tr>
<tr>
<td>straw: cereals</td>
<td></td>
</tr>
<tr>
<td>legumes</td>
<td>1.1</td>
</tr>
<tr>
<td>seeds: wheat, barley, oat</td>
<td>1.5 - 2.2</td>
</tr>
<tr>
<td>rice</td>
<td>1.3</td>
</tr>
<tr>
<td>maize</td>
<td>1.5</td>
</tr>
<tr>
<td>millet</td>
<td>1.8</td>
</tr>
<tr>
<td>pea</td>
<td>3.5</td>
</tr>
<tr>
<td>peanut</td>
<td>4.1</td>
</tr>
<tr>
<td>bean</td>
<td>4.3</td>
</tr>
<tr>
<td>soybean</td>
<td>5.4</td>
</tr>
<tr>
<td><strong>ANIMALS</strong></td>
<td></td>
</tr>
<tr>
<td>milk (cow)</td>
<td>0.56</td>
</tr>
<tr>
<td>serum</td>
<td>1.2</td>
</tr>
<tr>
<td>blood</td>
<td>3.2</td>
</tr>
<tr>
<td>whole body</td>
<td>8 - 10</td>
</tr>
<tr>
<td>meat</td>
<td>13</td>
</tr>
</tbody>
</table>
b) Add 5-10 ml conc. sulfuric acid to the plant material in the Kjeldahl flask. Material with high amount of carbohydrates needs more sulfuric acid (Table 3). All plant material should be in contact with $\text{H}_2\text{SO}_4^+$, with no dry material remaining on the glass walls of the flask.

c) Heat gently (~ 250°C) for 10 minutes, turning the flask periodically.

d) Add 0.7-1.4 g selenium reaction mixture (Wieninger mixture: 500 parts $\text{Na}_2\text{SO}_4$ + 8 parts $\text{CuSO}_4$ + 8 parts Se). Mix the content.

e) Continue the heating (ideal temperature for boiling: not higher than 410°C but not below the boiling point of concentrated $\text{H}_2\text{SO}_4$ ~ 340°C) until the digest becomes colourless after cooling down to room temperature. (In case that the digest does not become colourless, add more selenium reaction mixtures or add, after cooling, some drops of $\text{H}_2\text{O}_2$ 30%)

f) Continue the gentle boiling at 340°C - 410°C for 45 min.

A modified method without Se-reaction mixture can be used in laboratories which are situated in altitudes below 2000 m. In this case one can use 30% $\text{H}_2\text{O}_2$ only for sample oxidation. This is performed by adding very carefully few drops of 30% $\text{H}_2\text{O}_2$ to the $\text{H}_2\text{SO}_4$ extract, which was before cooled down on a sand bath to ~ 120-140°C. This is followed by heating again to boiling temperature of concentrated $\text{H}_2\text{SO}_4$, and the boiling is continued for ~ 5 minutes. Repeat dropwise addition of $\text{H}_2\text{O}_2$ until the digest becomes colourless at room temperature. After the last addition of $\text{H}_2\text{O}_2$ the gentle boiling has to be continued for 30 minutes.

For samples which contain nitrate or nitrite, e.g. soil samples, procedure i) cannot be used. The addition of concentrated $\text{H}_2\text{SO}_4$ would decompose those compounds and release gaseous nitrogen oxides ($\text{NO}_x$) which will lead to nitrogen losses. In this case the samples have to be pretreated. Amongst the existing methods, i.e. the alkaline reduction method, the salicylic acid method and the potassium permanganate-iron method, we prefer to use the last one in most cases.
Table 3: C/N ratio of various materials

<table>
<thead>
<tr>
<th>Biological materials</th>
<th>C/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straw: wheat and rye</td>
<td>75 - 100</td>
</tr>
<tr>
<td>oat and barley</td>
<td>55 - 70</td>
</tr>
<tr>
<td>Peat</td>
<td>30 - 50</td>
</tr>
<tr>
<td>Farm manure</td>
<td>15 - 25</td>
</tr>
<tr>
<td>Green plants</td>
<td>10 - 25</td>
</tr>
<tr>
<td>Top soil</td>
<td>9 - 13</td>
</tr>
<tr>
<td>Subsoil</td>
<td>6 - 9</td>
</tr>
<tr>
<td>Amino acids</td>
<td>5 (1.3 - 13)</td>
</tr>
</tbody>
</table>

(ii) The potassium permanganate-iron pretreatment for samples with nitrite (NO$_2^-$) and nitrates (NO$_3^-$):

a) Place an air dried soil sample containing about 1 mg N in a dry 100 ml Kjeldahl flask. (For analyses one can use as well moist soil samples but the nitrogen data have then to be corrected for the moisture content of the soil!)

b) Add 4 ml of 2.5% KMnO$_4$ solution and swirl from time to time during 5 minutes.

c) Add 15 ml distilled water and 5 ml conc. H$_2$SO$_4$, swirl from time to time during 30 minutes, all nitrites (NO$_2^-$) should now be converted to nitrates (NO$_3^-$).

d) Add 2-3 pumic stones and boil gently for 1 hour, add 100 mg portions of reduced Fe at a time, every 10 minutes. Repeat 5 times the addition of Fe. After the last addition boil for 10 minutes and cool the flask. All NO$_3^-$ should now be converted to NH$_4^+$.

e) Add 2-3 ml conc. H$_2$SO$_4$, swirl, add selenium reaction mixture as described in previous Kjeldahl procedure i) d). Keep the solution
boiling until H₂O is removed and foaming ceases. Increase the temperature to 410°C and continue heating until the digest becomes colourless (~ 2 hours).

In general the critical factors for the digestions are: temperature, catalyst, time, reflux and decomposition of ammonia catalyst complex. For further information see list of references.

2) Distillation:

In this step the ammonium salts are separated from the concentrated H₂SO₄. In strong alkaline solution gaseous ammonium can be distilled and trapped in standard acid solution. The following reactions take place:

\[(\text{NH}_4)_2\text{SO}_4 + 2\text{NaOH} \rightarrow 2\text{NH}_3 + \text{Na}_2\text{SO}_4 + 2\text{H}_2\text{O}\]

\[\text{NH}_3 + \text{HCl} \rightarrow \text{NH}_4\text{Cl} + \text{HCl} \text{ excess}\]

Dilute the Kjeldahl extract with distilled water. The volume of H₂O depends on the distillation apparatus used. For steam distillation apparatus usually less water is added than for direct boiling of the diluted digest on heaters. In order to make sure that the added amount of 50% NaOH is in excess (to make the solution strongly alkaline) 2-3 drops of an indicator (e.g. Tashiro indicator: 1g Methylred + 0.5g Methyleneblue dissolved in 200 ml 70% ethanol) is added. The addition of 50% NaOH to the diluted digest should be done very slowly, and the mixing of these two solutions can only be done when the system of distillation apparatus is closed, because there will be a sudden release of NH₃ as soon as the solution becomes alkaline! A titration flask with a precisely measured amount of standard acid (e.g. 0.1 N HCl) is placed under the condenser, the tip of the condenser should dip into the receiver acid in order to avoid any ammonia losses. For plant material 25 ml of 0.1 N HCl acid will be an adequate amount, whilst for soil samples more diluted acid is needed, e.g. 15-20 ml 0.02 N HCl to trap quantitatively the liberated NH₃ and have still some acid in excess. The distillation time depends on the size of apparatus and sample.

In this procedure standard HCl or H₂SO₄ have been used. Many Kjeldahl procedures suggest boric acid as receiving reagent for ammonia. In the case, the Kjeldahl extract is being prepared not only for total nitrogen analyses but as well for ¹⁵N/¹⁴N ratio analyses by emission or mass spectrometer method, boric acid would cause difficulties and in some instances even interferences.
3) Total nitrogen determination by volumetric method (back-titration of standard acid with standard sodium hydroxid):

Reactions:

a) \( \text{NH}_3 + \text{HCl} \rightarrow \text{NH}_4\text{Cl} + \text{HCl} \) excess
b) \( \text{HCl} \) excess + \( \text{NaOH} \) \( \rightarrow \) \( \text{NaCl} + \text{H}_2\text{O} \)

Reaction a) shows that 1 mol \( \text{NH}_3 \), which contains 1 atom N, reacts with 1 mol HCl. The equivalent of nitrogen for 1 ml 0.1 N HCl = 1.4 mg N, in other words 1 ml of 0.1 N HCl, which was used for neutralizing the ammonia is equal to 1.4 mg nitrogen in the digested sample (atom weight of nitrogen = 14 g).

Calculation exercise for total N:

\( A = 20.00 \) ml of 0.1 N HCl was pipetted to the receiver flask.

\( B = 9.45 \) ml of 0.1 N NaOH was used for backtitration to determine the excess of 0.1 N HCl.

\( A_1 = A - B = 10.55 \) ml 0.1 N HCl used for neutralization of \( \text{NH}_3 \).

\( T = \) ml 0.1 N HCl used in a blank (e.g. 0.05). A blank must be run periodically with the reagent used for digestion and distillation procedures. This blank has to be very low, otherwise the nitrogen present in the reagents would dilute the \( ^{15}\text{N} \) of the samples and accuracy of \( ^{15}\text{N} / ^{14}\text{N} \) sample ratio determination will be considerably affected. It is very difficult to correct for these nitrogen contaminations.

\( W = \) weight of the sample in mg (e.g. 527)

\[
\% \text{N} = (A_1 - T) \times \frac{1.4 \times 100}{W}
\]

\[
\% \text{N} = (10.55 - 0.055) \times \frac{1.4 \times 100}{527} = \frac{2.79}{2.79}
\]

4) Addition of acid

To avoid nitrogen losses for later \( ^{15}\text{N} / ^{14}\text{N} \) ratio analyses the titrated extract has to be acidified immediately after titration.
5) Conversion of ammonium salts to nitrogen gas

The ammonium compounds present in this extract are now converted to nitrogen gas, using the Rittenberg-reaction. For this reaction an alkaline sodiumhypobromite solution is used as oxidizing reagent in an evacuated reaction vessel with an adequate amount of Kjeldahl extract, depending on the $^{15}$N/$^{14}$N analyser system. The following reaction takes place:

$$2\text{NH}_4\text{Cl} + 3\text{NaOBr} + 2\text{NaOH} \rightarrow \text{N}_2 + 5\text{H}_2\text{O} + 3\text{NaBr} + 2\text{NaCl}$$

There are automatic Kjeldahl apparatuses available in which digestion, distillation and titration or colorimetric assay are performed in a sequence. However, one can as well use very simple equipment such as serial heaters or block digesters, simple distillation units and burettes for titration. Since all these reactions take place in solution, serious problems of contamination can occur. The following cases of interference to $^{15}$N/$^{14}$N ratio determinations, associated with digestion and distillation, are most frequent:

a) Dilution or contamination from outside sources such as absorption of ammonia or amines from the air (atmosphere). In other words, any acid solution traps ammonia, which dilutes the $^{15}$N-nitrogen of the sample.

b) Nitrogen impurities of chemicals.

c) Dissolved air in the solutions.

d) Cross contamination from poorly cleaned laboratory equipment, especially the distillation apparatus.

e) Cross contamination in automatic Kjeldahl equipment, since these apparatuses are not designed to use the solution after titration (or colorimetric assay) for further analyses, there might occur a carry-over between samples.

**Dumas-dry combustion method**

In this method all nitrogen compounds, independent whether they are organic or inorganic, ammonium or nitrate nitrogen, will be converted in only one step to $\text{N}_2$-gas. The reaction takes place on dry material, at high temperature (above 450°C) in a closed, nitrogen free atmosphere, using copper oxide (CuO) and copper (Cu) as oxidizing and reducing reagents, respectively,
and CaO to absorb water and CO₂. The following possible reactions occur, which serve as examples:

\[
\begin{align*}
2\text{NH}_4\text{Cl} + 4\text{CuO} & \rightarrow 450^\circ \text{C} \quad \text{N}_2 + 4\text{H}_2\text{O} + 2\text{CuCl} + 2\text{Cu} \\
2\text{KNO}_3 + 5\text{Cu} & \rightarrow 450^\circ \text{C} \quad \text{N}_2 + \text{K}_2\text{O} + 5\text{CuO} \\
\text{CO(NH}_2)_2 + 3\text{CuO} & \rightarrow 450^\circ \text{C} \quad \text{N}_2 + \text{CO}_2 + 2\text{H}_2\text{O} + 3\text{Cu}
\end{align*}
\]

The CO₂ (carbon dioxide) and H₂O (water) formed during combustion are absorbed on calcium oxide (CaO) which is added to the CuO/Cu mixture.

\[
\begin{align*}
\text{CaO} + \text{H}_2\text{O} & \rightarrow \text{Ca(OH)}_2 \\
\text{CaO} + \text{CO}_2 & \rightarrow \text{Ca CO}_3
\end{align*}
\]

When the reaction is completed and the system has reached room temperature only pure N₂ gas is present for total nitrogen and $^{15}\text{N}/^{14}\text{N}$ ratio determination. Presently there are several nitrogen analysers available commercially, which are using these principles of chemical reactions. The nitrogen gas generated in these analysers can be used for $^{15}\text{N}/^{14}\text{N}$ ratio analyses by mass-spectrometers. Since this method is using solid material and the reactions take place in a closed system, dilution and cross contamination risks for $^{15}\text{N}$-nitrogen of the sample are much smaller than in the Kjeldahl-Rittenberg method.

It is as well possible to combine the Kjeldahl procedure with the Dumas method instead of using the Rittenberg conversion. In this case the slightly acid solution after titration has to be evaporated to dryness, in order to generate N₂-gas from NH₄ salts using CuO and CaO for a dry combustion.

REFERENCES


METHODS FOR $^{15}$N DETERMINATION

H. AXMANN
Soil Science Unit,
FAO/IAEA Programme,
Agency's Laboratory,
International Atomic Energy Agency,
Seibersdorf

PRINCIPLE OF DETECTION

The $^{14}N/^{15}N$ ratio of nitrogen gas generated from a sample can be determined by mass spectrometry or emission spectrometry.

The $^{14}N$ and $^{15}N$ atoms in nitrogen gas are paired to form molecules $^{14}N-^{14}N$, $^{14}N-^{15}N$ and $^{15}N-^{15}N$, which can be written as $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$. Both mass and emission spectrometric methods provide output signals which are proportional to the number of these three types of molecules.

In any nitrogen gas mixture, the atom % $^{15}N$ abundance (a) can be derived from the following equation:

$$a = \left\{ \frac{30}{28} \frac{29}{30} \right\} \times 100$$

(1)

which represents the ratio of the nitrogen 15-atoms to the total number of nitrogen atoms expressed in percentage.

The % $^{15}N$ abundance (a) of a sample can be calculated by inserting the output signals for $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ molecules as measured by mass or emission spectrometers into the above equation. If equilibrium exist between the $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ molecules according to $^{28}N_2 + ^{30}N_2 \leftrightarrow 2^{29}N_2$ it becomes unnecessary to measure the output signals of all the three nitrogen molecules; only the $^{28}N_2$ and $^{29}N_2$ signals need to be measured.

The atom % $^{15}N$ abundance (a) can then be calculated from the following equation:

$$\text{atom %}^{15}N \text{ abundance} = \frac{100}{2R + 1}$$

(2)
where $R$ is the ratio of the intensities of $^{28}\text{N}$ and $^{29}\text{N}$ signals:

$$R = \frac{I(^{28}\text{N})}{I(^{29}\text{N})}$$  \hspace{1cm} (3)

**PRINCIPLE OF MASS SPECTROMETRY**

A mass spectrometer designed for gas analyses essentially comprises five units (Fig. 1).

1) An inlet system for the introduction of nitrogen gas, or a connection to an automatic nitrogen analyser,

2) An ion source, where the nitrogen molecules are bombarded with electrons, become charged and accelerated,

3) A magnetic field in which the charged molecules are separated into different paths according to their momentum.

4) A collector placed at the end of the flight tube, where the molecules are discharged, and the discharge currents are amplified,
5) A recorder, which register the amplified discharge currents.

The heights of the peaks are proportional to the amounts of the three N molecules in the gas mixture.

Although the principle of mass-spectrometry is simple, the practice is rather complicated. The whole system must be maintained under high vacuum (10^{-11}-10^{-12} torr) and the electronic components must be highly stabilized.

The sample size depends mainly on the volume of the inlet system and of the sample container. Therefore the range can vary between approximately 50 µg to 2 mg nitrogen/sample. The precision is very high and + 0.00001 % can be detected. Various types of mass spectrometer are available. Lists of some suppliers can be obtained from the authors.

PRINCIPLE OF EMISSION SPECTROMETRY

In emission spectrometry an external energy source is used to bring the nitrogen molecules to an exited state. The nitrogen molecules can incorporate extra energy in five ways, but only the following three are of interest for emission spectrometry:

1. raising one or more electrons to a higher orbital as in the case of atoms.
2. increasing vibration (oscillation) of the two atoms along the internuclear axis and
3. increasing the rotation of the molecule around an axis passing through the centre of gravity perpendicular to the nuclear axis.

When the exited molecules return to the ground state, the energy difference is emitted as electromagnetic radiation of distinct wavelengths. Even though the differences in wavelengths of the light emitted by the excited $^{28}\text{N}_2$, $^{29}\text{N}_2$ and $^{30}\text{N}_2$ molecules are very small (297.7 nm for $^{28}\text{N}_2$, 298.3 nm for $^{29}\text{N}_2$ and 298.9 nm for $^{30}\text{N}_2$) it can be resolved by a monochromator and the light intensities measured.

An emission spectrometer for $^{15}\text{N}$ ratio analyses consists of several units, shown in Fig. 2.
These units are:

1. Microwave (or high frequency) power generator for the excitation of the nitrogen gas in the sample tube,
2. An excitation stand which consists either of an antenna or a cavity in which the sample tube can be fixed for the transfer of energy from the generator to the nitrogen gas,
3. A monochromator to resolve the light of different wavelengths,
4. A photomultiplier and an amplifier for the conversion of the light quanta to electrical signals,
5. A recorder or digital integrator with a printer, to convert the electrical signals into readable records.

An emission spectrometer is much simpler than a mass spectrometer. High vacuum is not needed and the instrument can be installed, maintained and operated by any experienced analyst.

The sample size for an emission spectrometer analyser depends mainly on the volume and on preparation method of the discharge tube. It can vary from 0.2 µg N to 20 µg N.

The precision is lower than for a mass spectrometer. The older types can only measure ± 0.01 % whereas the modern types have ± 0.065 % accuracy. The range is usually between ~ 0.45 % - 80 % or 0.365 % to 80 %, for old or new types of emission spectrometers, respectively.
Table 1: Comparison of $^{15}$N analytical methods.

<table>
<thead>
<tr>
<th>Instrument and method</th>
<th>Emission spectrometer</th>
<th>Mass spectrometer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&quot;old&quot; type 1</td>
<td>&quot;new&quot; type = NOI-Be 2) with an inlet system</td>
</tr>
<tr>
<td>Material which can be used for analyses</td>
<td>Only kjeldahl extracts (NH$_4^+$ salts)</td>
<td>Any type of material (dry combustion)</td>
</tr>
<tr>
<td>Lowest amount of N required for the analysis</td>
<td>2 µg (0.2)</td>
<td>10 µg</td>
</tr>
<tr>
<td>Lowest at. % $^{15}$N which can be measured</td>
<td>0.55 (0.45)</td>
<td>Near to natural abundance 0.36</td>
</tr>
<tr>
<td>Rel. standard deviation</td>
<td>~± 2–3%</td>
<td>~±0.6–1%</td>
</tr>
<tr>
<td>Total time for analyses in min.</td>
<td>30 – 40</td>
<td>2</td>
</tr>
<tr>
<td>Time for measurement only, in min.</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Time for sample-preparation in min.</td>
<td>25 – 35</td>
<td>0</td>
</tr>
<tr>
<td>Cost of equipment in US $</td>
<td>~ 35.000</td>
<td>~45.000–50.000</td>
</tr>
</tbody>
</table>

1) Only sealed discharge tubes can be used for measurements.
2) No preparation of discharge tubes is needed; the instrument has a flow through discharge tube; N$_2$ gas is generated from Kjeldahl extract and Sodiumhypobromide solution.
ISOTOPE TECHNIQUES IN SOIL FERTILITY
AND PLANT NUTRITION STUDIES

F. ZAPATA
Soil Science Unit,
FAO/IAEA Programme,
Agency's Laboratory,
International Atomic Energy Agency,
Seibersdorf

INTRODUCTION

Fertilizers are one of the essential inputs which have to be used for maintaining and/or increasing the soil fertility level in intensive agricultural systems. The purpose of applying fertilizers is primarily to supply the crop with essential plant nutrients. The major plant nutrients (N, P and K) have to be applied regularly to compensate for the amounts exported from the soil by the harvested plant parts. Other plant nutrients such as Ca, Mg, S and the microelements also need to be added to maintain adequate levels of these nutrients or to correct deficiencies (FAO, 1983a, 1983b, 1984 and 1985).

Fertilizers are applied to facilitate plant uptake of a particular nutrient. Increased uptake can lead to a yield response if the nutrition is a limiting factor. It is important though to note that the fertilizer is not applied to obtain a yield response but to feed the plant. The yield response is a consequence of the additional uptake of the nutrient when other production factors are adequate (Broeshart, 1974; Fried, 1978b).

A combination of all the production factors and conditions in an agricultural system results in a given yield and only if all factors are optimized (fertilizer, soil, plant, water, pest control, etc.) will yield be maximized. In fact the contribution of fertilizer to increased yield is perhaps the greatest among the purchased inputs. Fertilizer, when used in combination with the other adequate inputs such as high-yielding varieties and irrigation water, can result in positive interaction thereby increasing further its contribution to increased yield (Fried, 1978b).

FERTILIZER USE EFFICIENCY

What is fertilizer use efficiency?

Fertilizer use efficiency is a quantitative measure of the actual uptake of fertilizer nutrient by the plant in relation to the amount of nutrient added to the soil. A common form of expression of fertilizer use efficiency
is plant recovery or % utilization of the added fertilizer. This is shown in equation (1):

\[
\text{% utilization of added fertilizer} = \frac{\text{amount of nutrient in the plant derived from the fertilizer}}{\text{amount of nutrient applied as fertilizer}} \times 100
\]  

The concept of fertilizer use efficiency, however, is much broader. It implies not only the maximum uptake of the applied nutrient by the crop but also the availability of the applied nutrient under variable climatic and edaphic conditions. Environmental aspects such as pollution resulting from the fertilizer application are also considered. It is important to study the efficient use of fertilizers because we are interested to obtain the highest possible yield with a minimum fertilizer application.

The crop responds to the application of nutrients such as nitrogen and phosphorus when the soil is deficient in such nutrients. It is the objective to apply the fertilizer to the crop, not to the soil and to avoid that the fertilizers become unavailable to the crop, i.e. fixed in the case of phosphorus or lost as nitrate by leaching or as gaseous losses due to denitrification and/or volatilization in the case of nitrogen.

It is therefore essential to ensure that the applied fertilizer is taken up by the crop to the highest possible extent. This is done after assessing the best fertilizer practices such as sources, timing, placement and their interactions under different farming systems (FAO, 1980).

**How to measure fertilizer use efficiency?**

The best combination of fertilizer practices can be established for each crop by carrying out field experiments under different environmental conditions. When these experiments have been conducted with identical design and layout, it is possible to determine what generalization can be made with respect to placement, timing and source of fertilizer that result in the highest fertilizer uptake by the crop.

Therefore in practice we carry out a series of carefully designed field experiments in several locations over a period of time for estimating the effect of placement, timing and source on fertilizer nutrient uptake. Of course for the farmer only the yield matters but it is equally important that this yield is obtained with a minimum of fertilizer investment (minimum cost).
The following methods can be utilized to assess the effect of fertilizer practices:

a) The classical or conventional method measures the biological response or the effect of increasing fertilizer rates on crop yield. Yield is however dependent on a series of factors: some controllable, others non-controllable ones. They all will influence to a variable extent the yield's quantity and quality.

b) Methods based on the nutrient uptake.

1) Difference method: In this indirect method the nutrient uptake of the crop from the control plot (without fertilizer application) is subtracted from that of the fertilized treatments. It is assumed that the nutrient uptake of the control plot measures the amount of nutrient available from the soil, whereas that of the fertilized treatments, the amounts of nutrients available from soil and fertilizer. This method furthermore assumes that all nutrient transformations i.e. mineralization, immobilization and other processes in the case of nitrogen, are the same for both fertilized and unfertilized soils. Obviously, this is an erroneous assumption, and can account for gross differences between recoveries calculated by non-isotope and isotopic methods (Broeshart, 1974; Westerman and Kurtz, 1974; Harmsen and Moraghan, 1988).

2) Isotopic method: The only direct means of measuring nutrient uptake from the applied fertilizer is by the use of isotopes. Extensive work has been conducted using N-fertilizers labelled with the stable isotope $^{15}\text{N}$ and P-fertilizers labelled with the radioactive isotopes $^{32}\text{P}$ or $^{33}\text{P}$. This does not mean that K and the other plant nutrients are not important. However, for K there is no suitable isotope available for field experimentation. Furthermore by initiating work with N and P utilizing isotopic methods it was envisaged that corresponding studies would essentially be conducted with the others, as researchers in developing countries became experienced and confident with the methodology (Broeshart, 1974; Fried, 1978b; IAEA, 1970a, 1970b, 1971, 1974, 1975, 1978a, 1980, 1983; FAO, 1980).

Table 1 contains the principal tracer isotopes used in soil-plant relationships studies. The chemical elements have been grouped into 3 categories. The first two groups refer to the essential plant
nutrients i.e. macro and micronutrients, respectively, while the third one consists of a miscellaneous group of trace elements and others used in soil-plant relationships and related studies.

It is often argued that the labelled fertilizers lose their identity in the soil since they become incorporated into the organic matter, soil solution, ion exchange processes, etc. resulting in just one pool of nutrients. The only basic assumption made when utilizing isotopically labelled fertilizer is that the behaviour of the isotope and the carrier is identical in the soil-plant system. In other words there should not be any isotope effect.

The isotopic labelling of the fertilizer is best done during the manufacturing process by specialized firms.

*Text cont. on p. 73.*
<table>
<thead>
<tr>
<th>Element</th>
<th>Most abundant isotope</th>
<th>Tracer isotope</th>
<th>Characteristics</th>
<th>Typical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>$^12C$</td>
<td>$^11C$</td>
<td>R, T $1/2 = 20.5$ m</td>
<td>Limited because of short half-life.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{12}C$</td>
<td>$\beta$ emitter (1 MeV)</td>
<td>C-12 enriched (C-13 depleted) material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{13}C$</td>
<td>$S$, natural abundance = 98.892%</td>
<td>Organic reaction mechanisms work.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{14}C$</td>
<td>$S$, natural abundance = 1.108% Detection of $^{13}C/^{12}C$ ratio by MS</td>
<td>Soil organic matter studies in ecosystems</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{14}C$</td>
<td>$R, T 1/2 = 5720$ y $\beta$ emitter (0.156 MeV)</td>
<td>Photosynthesis, C translocation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{2}H$</td>
<td>$S$, natural abundance = 0.01492% Detection of $^{2}H/^{1}H$ ratio by MS</td>
<td>Water movement, biochemical studies</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>$^{1}H$</td>
<td>$^{2}E$</td>
<td>$S$, natural abundance = 0.01492% Detection of $^{2}H/^{1}H$ ratio by MS</td>
<td>Water movement, metabolism.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{3}H$</td>
<td>$R, T 1/2 = 12.3$ y Very weak $\beta$ emitter (0.0181 MeV)</td>
<td>Water movement, metabolism.</td>
</tr>
<tr>
<td>Oxygen</td>
<td>$^{16}O$</td>
<td>$^{16}O$</td>
<td>$S$, natural abundance = 99.759%</td>
<td>Photosynthesis, respiration.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{17}O$</td>
<td>$S$, natural abundance = 0.037%</td>
<td>Soil organic matter studies.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^{18}O$</td>
<td>$S$, natural abundance = 0.204% Detection of the oxygen isotope ratio by MS</td>
<td>Ecological studies. Hydrology.</td>
</tr>
<tr>
<td>Element</td>
<td>Most abundant isotope</td>
<td>Tracer isotope</td>
<td>Characteristics</td>
<td>Typical Applications</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>--------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Nitrogen     | $^{14}_N$             | $^{13}_N$      | R, $T_{1/2} = 10$ m  
β emitter (1.2 MeV) and  
γ (0.511 MeV)              | Limited because of short half-life.  
Very short-term studies on N$_2$ fixation, denitrification |
|              | $^{14}_N$             |                | S, natural abundance = 99.634%  
Detection of $^{15}_N/^{14}_N$ ratio by MS | N-14 enriched (N-15 depleted) materials for single season fertilizer use efficiency studies |
|              | $^{15}_N$             |                | S, natural abundance = 0.366%  
Detection of $^{15}_N/^{14}_N$ ratio either by MS or ES | Fertilizer N use efficiency, biological nitrogen fixation, N balance, N transformation in soils, N availability from organic-matериалs, animal nutrition studies |
| Phosphorus   | $^{31}_P$             | $^{32}_P$      | R, $T_{1/2} = 14.3$ d  
β emitter (1.71 MeV)  
GM, Cerenkov or LSC | Fertilizer P use efficiency, residual P fertilizer studies.  
Exchangeable P in soils.  
Root activity patterns of crops.  
Root distribution in soils.  
Agronomic evaluation of rock phosphates.  
Residual P fertilizer availability. |
|              | $^{33}_P$             |                | R, $T_{1/2} = 25$ d  
β emitter (0.248 MeV)  
LS counting             | Root autoradiography.  
Diffusion in soil.  
Double labelling for root activity patterns.  
Fertilizer P use efficiency. |
| Potassium    | $^{39}_K$             | $^{40}_K$      | R, $T_{1/2} = 1.3 \times 10^3$ γ  
β (1.3 MeV) LSC, Cerenkov  
γ (1.46 MeV) emitter NaI (Tl)  
Natural Radioisotope, Natural abundance = 0.0118% | Exchangeable K in soils. |
|              | $^{41}_K$             |                | S, natural abundance = 6.77%             | Potentially useful. |
|              | $^{42}_K$             |                | R, $T_{1/2} = 12.4$ h  
β (3.5 and 2.6 MeV) LSC, Cerenkov  
γ (1.52 MeV) emitter NaI (Tl) 2"/3" | Potentially useful.  
Ion uptake mechanisms. Limited because of short half-life. |
<table>
<thead>
<tr>
<th>Element</th>
<th>Most abundant isotope</th>
<th>Tracer isotope</th>
<th>Characteristics</th>
<th>Typical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>40Ca</td>
<td>86Rb</td>
<td>R, T 1/2 = 18.7 d&lt;br&gt;β (1.8 and 0.7 MeV) Cerenkov, LSC&lt;br&gt;and γ (1.08 MeV)&lt;br&gt;Counting by GM or LSC NaI (Tl) 2&quot;/3&quot;</td>
<td>Substitute tracer for R. Only qualitative studies like placement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45Ca</td>
<td>R, T 1/2 = 165 d&lt;br&gt;β emitter (0.252 MeV)&lt;br&gt;Counting by LSC</td>
<td>Soil Ca (ion uptake, exchangeable Ca) and plant Ca movement (root's autoradiography)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>85Sr</td>
<td>R, T 1/2 = 64 d&lt;br&gt;γ emitter (0.514 MeV)&lt;br&gt;Counting by LSC, Cerenkov</td>
<td>Cation exchange capacity of soil&lt;br&gt;Ion uptake mechanisms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>89Sr</td>
<td>R, T 1/2 = 52.7 d&lt;br&gt;β emitter (1.463 MeV)&lt;br&gt;Counting by LSC, Cerenkov</td>
<td>Substitute tracer for Ca</td>
</tr>
<tr>
<td>Magnesium</td>
<td>24Mg</td>
<td>26Mg</td>
<td>S, natural abundance = 11.29%</td>
<td>Potentially useful. Environmental pollution.&lt;br&gt;Ecological and medical research</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28Mg</td>
<td>R, T 1/2 = 21.3 h&lt;br&gt;β emitter (0.5 MeV) and also γ emitter (0.03; 0.4; 0.95; 1.35 MeV)</td>
<td>Movement in plants.</td>
</tr>
<tr>
<td>Sulfur</td>
<td>32S</td>
<td>34S</td>
<td>S, natural abundance = 4.25%</td>
<td>Potentially useful. Environmental pollution.&lt;br&gt;Ecological and medical research</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35S</td>
<td>R, T 1/2 = 87 d&lt;br&gt;β emitter (0.165 MeV)&lt;br&gt;Counting by LSC</td>
<td>Uptake from atmosphere (SO_2).&lt;br&gt;Cycling in pastures.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Availability from soil.</td>
</tr>
<tr>
<td>Element</td>
<td>Most abundant isotope</td>
<td>Tracer isotope</td>
<td>Characteristics</td>
<td>Typical Applications</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------</td>
<td>---------------</td>
<td>----------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Iron</td>
<td>$^{56}\text{Fe}$</td>
<td>$^{55}\text{Fe}$</td>
<td>$R, T \frac{1}{2} = 2.6 \gamma$ &lt;br&gt; Electron capture (EC) &lt;br&gt; Counting by LSC</td>
<td>Fertility erosion</td>
</tr>
<tr>
<td></td>
<td>$^{59}\text{Fe}$</td>
<td></td>
<td>$R, T \frac{1}{2} = 45.6 \text{ d}$ &lt;br&gt; $\beta$ emitter (0.475; 0.273 MeV) LSC &lt;br&gt; $\gamma$ emitter (1.1; 1.29 MeV) NaI (Tl) $2^\text{m}/3^\text{m}$</td>
<td>Soil and plant movement</td>
</tr>
<tr>
<td>Copper</td>
<td>$^{63}\text{Cu}$</td>
<td>$^{64}\text{Cu}$</td>
<td>$R, T \frac{1}{2} = 12.8 \text{ h}$ &lt;br&gt; $\beta$ emitter (0.6; 0.7 MeV) and $\gamma$ emitter (1.34 MeV) from EC</td>
<td>Complexing in soil solution</td>
</tr>
<tr>
<td></td>
<td>$^{65}\text{Cu}$</td>
<td></td>
<td>$S, \text{ natural abundance} = 30.9%$</td>
<td>Animal nutrition studies. Potentially useful</td>
</tr>
<tr>
<td></td>
<td>$^{67}\text{Cu}$</td>
<td></td>
<td>$R, T \frac{1}{2} = 58.5 \text{ h}$ &lt;br&gt; $\beta$ emitter (0.58; 0.48; 0.40, 0.091 MeV) and $\gamma$ emitter (0.092; 0.184 MeV)</td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>$^{55}\text{Mn}$</td>
<td>$^{52}\text{Mn}$</td>
<td>$R, T \frac{1}{2} = 5.7 \text{ d}$ &lt;br&gt; $\beta$ emitter (0.6 MeV) and $\gamma$ emitter (1.43; 0.94; 0.74; 0.84 MeV)</td>
<td>Complexing in soil solution</td>
</tr>
<tr>
<td></td>
<td>$^{54}\text{Mn}$</td>
<td></td>
<td>$R, T \frac{1}{2} = 314 \text{ d}$ &lt;br&gt; $\gamma$ emitter (0.835 MeV) and EC</td>
<td>Availability from soil. Soil and plant movement</td>
</tr>
<tr>
<td>Zinc</td>
<td>$^{64}\text{Zn}$</td>
<td>$^{65}\text{Zn}$</td>
<td>$R, T \frac{1}{2} = 245 \text{ d}$ &lt;br&gt; $\beta$ emitter (0.327 MeV) and $\gamma$ emitter (1.115 MeV) from EC</td>
<td>Complexing in soil solution. Availability from soil and fertilizer. Soil and plant movement</td>
</tr>
<tr>
<td>Element</td>
<td>Most abundant isotope</td>
<td>Tracer isotope</td>
<td>Characteristics</td>
<td>Typical Applications</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------</td>
<td>----------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Boron</td>
<td>$^{11}\text{B}$</td>
<td>$^{10}\text{B}$</td>
<td>$S$, natural abundance = 19.7%</td>
<td>Foliar absorption. Neutron activation. Neutron moderation (soil moisture studies).</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>$^{96}\text{Mo}$</td>
<td>$^{99}\text{Mo}$</td>
<td>$R, T \frac{1}{2} = 66.7 \text{ h}$ and $\beta$ emitter (1.2; 0.45 MeV) and $\gamma$ emitter (0.74; 0.18; 0.78; 0.37 and 0.041 MeV)</td>
<td>Plant nutrition.</td>
</tr>
<tr>
<td>III- OTHER ELEMENTS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorine</td>
<td>$^{35}\text{Cl}$</td>
<td>$^{35}\text{Cl}$</td>
<td>$S$, natural abundance = 75.53%</td>
<td>Solute movement in soils.</td>
</tr>
<tr>
<td></td>
<td>$^{36}\text{Cl}$</td>
<td></td>
<td>$R, T \frac{1}{2} = 3.08 \times 10^5 \text{ y}$ and $\beta$ emitter (0.714; 0.115 MeV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Counting by LSC</td>
<td></td>
</tr>
<tr>
<td>Iodine</td>
<td>$^{127}\text{I}$</td>
<td>$^{125}\text{I}$</td>
<td>$R, T \frac{1}{2} = 60 \text{ d}$ and $\beta$ emitter (0.61; 0.25; 0.81 MeV) and $\gamma$ emitter (0.36; 0.06; 0.72 MeV)</td>
<td>Herbicidal and insecticidal effects on life forms, water, air and soil.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\gamma$ (0.035 MeV), NaI (Tl) 2&quot; and X-ray (0.027 MeV), MLSC</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>$^{23}\text{Na}$</td>
<td>$^{22}\text{Na}$</td>
<td>$R, T \frac{1}{2} = 2.6 \text{ y}$ and $\beta$ emitter (0.5 MeV) LSC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\gamma$ (1.27 MeV) emitter NaI (Tl) 2&quot;/3&quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$R, T \frac{1}{2} = 15 \text{ h}$ and $\beta$ emitter (1.4 MeV) LSC, Cerenkov</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\gamma$ (2.75; 1.35 MeV) emitter NaI (Tl) 2&quot;/3&quot;</td>
<td></td>
</tr>
<tr>
<td>Element</td>
<td>Most abundant isotope</td>
<td>Tracer isotope</td>
<td>Characteristics</td>
<td>Typical Applications</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>---------------------</td>
</tr>
</tbody>
</table>
| Cobalt  | 59\(^{Co}\)          | 57\(^{Co}\)    | R, T 1/2 = 270 d  
EC conversion el. LSC  
\(\gamma\) emitter (0.122; 0.014 MeV)  
NaI (Tl) 2"/3" | Soil fertility erosion.  
(Spike for sediment's movement and deposition) |
|         | 58\(^{Co}\)          |                | R, T 1/2 = 71 d  
\(\beta\) emitter (0.48 MeV) LSC  
\(\gamma\) emitter (0.81; 1.64 MeV)  
NaI (Tl) 2"/3" | |
|         | 60\(^{Co}\)          |                | R, T 1/2 = 5.3 y  
\(\beta\) emitter (0.31 MeV) and  
\(\gamma\) emitter (1.33; 1.17 MeV) | |
| Cesium  | 133\(^{Cs}\)         | 134\(^{Cs}\)   | R, T 1/2 = 2.046 y  
\(\beta\) emitter (0.662; 0.089 MeV) and  
\(\gamma\) emitter (0.57; 0.605; 0.796 MeV) | Soil fertility erosion.  
(Radioactive fallout) |
|         |                      | 137\(^{Cs}\)   | R, T 1/2 = 30 y  
\(\beta\) emitter (1.176; 0.514 MeV) and  
\(\gamma\) emitter (0.662 MeV) | |
| Barium  | 138\(^{Ba}\)         | 131\(^{Ba}\)   | R, T 1/2 = 11.6 d  
EC and \(\gamma\) emitter (0.5; 0.122; 0.216 MeV) | |
|         |                      | 133\(^{Ba}\)   | R, T 1/2 = 7.5 y  
EC and \(\gamma\) emitter (0.082; 0.36; 0.30; 0.80 MeV) | |
|         |                      | 140\(^{Ba}\)   | R, T 1/2 = 12.8 d  
\(\beta\) emitter (1.02; 0.48 MeV) and  
\(\gamma\) emitter (0.54; 0.16 MeV) | |
<table>
<thead>
<tr>
<th>Element</th>
<th>Most abundant isotope</th>
<th>Tracer isotope</th>
<th>Characteristics</th>
<th>Typical Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>75As</td>
<td>73As</td>
<td>R, T 1/2 = 76 d&lt;br&gt;γ emitter (0.054; 0.14 MeV)</td>
<td>LSC, NaI (Tl) 2&quot;</td>
</tr>
<tr>
<td></td>
<td>74As</td>
<td></td>
<td>R, T 1/2 = 17.5 d&lt;br&gt;β emitter (0.9; 1.36 MeV) and&lt;br&gt;γ emitter (0.06; 0.64; 2.53 MeV)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>76As</td>
<td></td>
<td>R, T 1/2 = 26.0 h&lt;br&gt;β emitter (2.97; 2.41 MeV) and&lt;br&gt;γ emitter (0.56; 1.21; 0.66 MeV)</td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td>80Se</td>
<td>75Se</td>
<td>R, T 1/2 = 120 d&lt;br&gt;EC and γ emitter (0.265; 0.136; 0.280; 0.024; 0.58 MeV)&lt;br&gt;LSC, NaI (Tl) 2&quot;</td>
<td></td>
</tr>
<tr>
<td>Aluminium</td>
<td>27Al</td>
<td>26Al</td>
<td>R, T 1/2 = 7.4 x 10^5 y&lt;br&gt;β emitter (3.21; 1.16 MeV) and&lt;br&gt;γ emitter (1.83; 1.12 MeV)</td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>52Cr</td>
<td>51Cr</td>
<td>R, T 1/2 = 27.8 d&lt;br&gt;EC and γ emitter (0.32 MeV)&lt;br&gt;LSC, NaI (Tl) 2&quot;/3&quot;</td>
<td></td>
</tr>
<tr>
<td>Bromine</td>
<td>79Br</td>
<td>79Br</td>
<td>S, natural abundance = 50.54%</td>
<td>LSC, NaI (Tl) 2&quot;/3&quot;</td>
</tr>
<tr>
<td></td>
<td>81Br</td>
<td></td>
<td>S, natural abundance = 49.46%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>82Br</td>
<td></td>
<td>R, T 1/2 = 35.7 h&lt;br&gt;β emitter (0.44 MeV), LSC&lt;br&gt;γ emitter (0.55; 1.47 MeV),&lt;br&gt;NaI (Tl) 2&quot;/3&quot;</td>
<td></td>
</tr>
<tr>
<td>Element</td>
<td>Most abundant isotope</td>
<td>Tracer isotope</td>
<td>Characteristics</td>
<td>Typical Applications</td>
</tr>
<tr>
<td>----------</td>
<td>------------------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Mercury</td>
<td>$^{200}\text{Hg}$</td>
<td>$^{203}\text{Hg}$</td>
<td>$R, T \frac{1}{2} = 47 \text{ d}$ $\gamma$ emitter (0.3 MeV)</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td>$^{58}\text{Ni}$</td>
<td>$^{63}\text{Ni}$</td>
<td>$R, T \frac{1}{2} = 92 \text{ y}$ $\gamma$ emitter (0.067 MeV)</td>
<td></td>
</tr>
</tbody>
</table>

**Description**

- **R** = Radioactive isotope
- **S** = Stable isotope
- **T \frac{1}{2}** = Half-life expressed in time units, e.g., minutes (m), hour (h), day (d) and year (y)
- **B** = Beta radiation
- **$\gamma$** = Gamma radiation
- **EC** = Electron capture
- **LSC** = Liquid scintillation counting
- **MLSC** = Metal-loaded liquid scintillation counting
- **GM** = Geiger Müller
- **MS** = Mass spectrometry
- **ES** = Emission spectrometry
- **MeV** = Maximum energy's intensity in Mega-electron volts
In isotopic-aided fertilizer experiments, a labelled fertilizer is added to the soil and the amount of fertilizer nutrient which a plant has taken up is determined. In this way different fertilizer practices (placement, timing, sources, etc.) can be studied.

The first parameter to be determined when studying the fertilizer uptake by a crop by means of isotope techniques is the fraction of the nutrient in the plant derived from the (labelled) fertilizer, i.e.: \( \text{fdff} \). Often this fraction is expressed as percentage, i.e.: \( \% \text{dff} = \text{fdff} \times 100 \) \( (2) \)

The procedure followed in the calculation of this fraction and other derived parameters for nitrogen (stable isotope) and phosphorus (radioisotopes) using isotopically labelled materials is given below:

**Quantification of fertilizer N uptake**

The nitrogen isotope composition, i.e. the \( {}^{15}\text{N}/\text{total N} \) ratio, of any material can be expressed as atom % \( {}^{15}\text{N} \) (a) or simply % \( {}^{15}\text{N} \) abundance (see Axmann and Zapata, this volume). This isotopic ratio or % \( {}^{15}\text{N} \) abundance of a sample is measured directly in a single determination by optical emission or mass spectrometry. Since the % \( {}^{15}\text{N} \) natural abundance \( (a_0) \) is 0.336 atom % \( {}^{15}\text{N} \) this figure has to be subtracted from the % \( {}^{15}\text{N} \) abundance \( (a) \) of any enriched material to obtain the % \( {}^{15}\text{N} \) atom excess \( (\% {}^{15}\text{N} \text{ a.e.} = a') \).

What is then \( N_{\text{dff}} \)? It is the fraction of N in the plant derived from the \( {}^{15}\text{N} \) labelled fertilizer. From simple isotope dilution principle, the following relationship may be written:

\[
\frac{\% {}^{15}\text{N} \text{ a.e. plant sample}}{\% {}^{15}\text{N} \text{ a.e. labelled fertilizer}} = \frac{a' \text{ plant sample}}{a' \text{ labelled fertilizer}}
\]

or as a percentage

\[
\% N_{\text{dff}} = \frac{\% {}^{15}\text{N} \text{ a.e. plant sample}}{\% {}^{15}\text{N} \text{ a.e. labelled fertilizer}} \times 100
\]

Therefore, for the calculation of % \( N_{\text{dff}} \) it is necessary to determine the % \( {}^{15}\text{N} \) atom excess of the plant samples and of the fertilizer(s) used in the experiment.
For instance if Ndff = 0.25 this means that 1/4 of the nitrogen in the plant came from the fertilizer. In case that soil and fertilizer were the only sources of N available to the plant, then the remaining 3/4 of the nitrogen in the plant came from the soil. If these fractions are expressed in percentages the %Ndff = 25 % and %Ndfs = 75 %, where %Ndfs is % N derived from soil.

Exercise 1

In a field experiment 80 kg N/ha in the form of $^{15}$N labelled urea was applied to a maize crop. The maize was harvested at tasseling time with a dry matter yield of 4 tons/ha and a plant sample had 0.67 % $^{15}$N abundance and 3 % total N. The applied fertilizer had 1.37 % $^{15}$N abundance.

Questions:

1. What was the fraction of N in the plant, which was derived from the fertilizer or % Ndff?
2. What was the fraction of N in the plant, which was derived from the soil?
3. What was the total N yield of the crop?
4. What was the fertilizer N yield of the crop?
5. What was the fertilizer N utilization or recovery by the crop?

Calculations:

1. % N derived from the fertilizer

$^{15}$N atom excess plant $= 0.67 - 0.37 = 0.30$

$^{15}$N atom excess fertilizer $= 1.37 - 0.37 = 1.00$

The fraction of fertilizer N in the plant corresponding to 0.30 % $^{15}$N atom excess is calculated using equation 4:

$$\% \text{ Ndff} = \frac{0.30}{1.00} \times 100$$

$$\% \text{ Ndff} = 30 \%$$
2. \% N derived from the soil

Since the crop had only two sources of nutrients the \% N derived from the soil is obtained by difference as follows:

\[ \% N_{dfs} = 100 - \% N_{dff} \]

\[ 100 - 30 = 70 \% \]

3. The nitrogen yield of the crop

The total amount of N contained in the crop during the experimental period is obtained by recording the dry matter yield and multiplying it by the \% total N in the crop as follows:

\[ N \text{ yield} = 4000 \times \frac{3}{100} = 120 \text{ kg N/ha} \]

4. The fertilizer N yield of the crop

The amount of fertilizer N taken up by the crop is calculated by multiplying the total N yield by the fraction of Ndff:

\[ \text{Fertilizer N yield} = \frac{30}{100} \times 120 = 36 \text{ kg N/ha} \]

5. Fertilizer N utilization or recovery by the crop

The fraction of the fertilizer nutrient taken up by the plant in relation to the rate of fertilizer nutrient applied. It is commonly expressed as percentage:

\[ \% \text{Fertilizer N utilization} = \frac{36}{80} \times 100 = 45 \% \]

Exercise 2

In a field experiment, 60 kg N/ha as \( ^{15} \text{N} \) labelled ammonium sulphate were applied to hybrid sorghum. The \( ^{15} \text{N} \) treated plots were harvested at the grain milky stage of development. The harvest consisted of gathering all above-ground plant material in the harvesting area of the isotope plots and separating them into shoots and panicles. The fresh weight of each component was recorded. Adequate subsamples were taken and chemical and isotopic analyses were performed on each subsample separately.
Question:

What is the fertilizer N utilization of sorghum in this experiment?

Calculations:

The basic primary data are listed in the following table 2:

<table>
<thead>
<tr>
<th>Plant part</th>
<th>DM yield (tons/ha)</th>
<th>Total N (%)</th>
<th>N yield (kg/ha)</th>
<th>Ndff (%)</th>
<th>Fert. N yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoots</td>
<td>5.0</td>
<td>1.2</td>
<td>60</td>
<td>27.4</td>
<td>16.4</td>
</tr>
<tr>
<td>Panicles</td>
<td>2.2</td>
<td>2.1</td>
<td>46</td>
<td>19.8</td>
<td>9.1</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>106</td>
<td></td>
<td>25.5</td>
</tr>
</tbody>
</table>

1. As shown in the Table 2 N yield and fertilizer N yield of each plant part has to be calculated. Add up these data to obtain total N yield and total fertilizer N yield for the entire crop.

2. The next step is to calculate the weighed average % Ndff for the entire crop.

\[
\% \text{ Ndff (weighted average)} = \frac{25.5}{106} \times 100 = 24
\]

3. Finally the % fertilizer N utilization is calculated using the total fertilizer N uptake as follows:

\[
\% \text{ Fert. N utilization} = \frac{25.5}{60} \times 100 = 42.5
\]

Measurements needed for experiments with \( ^{15} \text{N} \)

In summary for all field and greenhouse experiments with \( ^{15} \text{N} \) (or any other stable isotope) labelled materials, the following basic primary data need to be recorded for each plot:

1. Dry matter (D.M.) yield for the whole plant or sub-divided into plant parts.
2. Total N content (% N in dry matter) of the whole plant or plant parts as in point 1. – This is done by chemical methods (Kjeldahl or another).
3. Plant % $^{15}$N abundance, which is analysed by emission or mass spectrometry.
4. Fertilizer % $^{15}$N abundance.
5. $^{15}$N labelled fertilizer(s) used and N rate(s) of application.

**Calculations for experiments with $^{15}$N**

1. % $^{15}$N abundance is transformed into % $^{15}$N atom excess by subtracting the natural abundance (0.3663 atom % $^{15}$N) from the % $^{15}$N abundance of the sample. Afterwards the following calculations can be made:

2. $%_{Ndff} = \frac{\%_{^{15}N \text{ atom excess plant}}}{\%_{^{15}N \text{ atom excess fertilizer}}} \times 100 \tag{5}$

3. Dry matter yield per unit area:

\[
DM \text{ yield (kg/ha)} = FW(kg) \times \frac{10000 \text{ (m}^2/\text{ha})}{\text{area harvested (m}^2\text{)}} \times \frac{SDW (kg)}{SFW (kg)} \tag{6}
\]

Where FW = fresh weight per area harvested and SDW and SFW are subsample dry and fresh weight, respectively.

4. N yield (kg/ha) = DM yield (kg/ha) \times \frac{\% N}{100} \tag{7}

5. Fertilizer N yield (kg/ha) = N yield (kg/ha) \times \frac{\% Ndff}{100} \tag{8}

6. % fertilizer N utilization: = \frac{\text{Fert. N yield}}{\text{Rate of N application}} \times 100 \tag{9}

**Quantification of fertilizer P uptake**

The phosphorus isotopic composition, i.e. the $^{32}$P/total P ratio, of any material is called specific activity (S.A.). The determination of the specific activity (S.A.) of a sample requires two independent measurements:

1) Determination of the activity (dpm or dps) of the radioisotope by radioassay techniques using appropriate detectors, i.e. proportional detector, Geiger Muller (GM) detector, liquid scintillation counting, Cerenkov
counting (for high-energy beta emitters) or sodium-iodide scintillation detectors.

2) Determination of the total nutrient content by any conventional chemical method, i.e. total P by spectrophotometric (methavanadate yellow) method.

It is customary to express the specific activities of plant samples and fertilizer in dpm $^{32}$P/g P or dps $^{32}$P/mg P, at the time the samples were counted.

It is important to note that the concept of specific activity (ratio $^{32}$P/total P) for radioisotopes is identical to that of % $^{15}$N atom excess (ratio $^{15}$N/total N) for stable isotopes.

What is Pdff? It is the fraction of P in the plant derived from the $^{32}$P or $^{33}$P labelled fertilizer material. It also follows from the isotope dilution principle that:

$$P_{dff} = \frac{S.A. \text{ plant sample}}{S.A. \text{ labelled fertilizer}}$$

or as a percentage:

$$\% \ P_{dff} = \frac{S.A. \text{ plant sample}}{S.A. \text{ labelled fertilizer}} \times 100$$

How is Pdff measured?

Both the activity and total P content in the plant and fertilizer samples should be determined as shown by Buchtela (this volume).

Exercise 3

An aliquot of a plant sample containing 8 mg P gives an activity of 800 dpm. From above, we have to determine the amount of P (g or mg P) in the plant derived from the fertilizer corresponding to 800 dpm. For this purpose the activity of a known amount of P from the labelled fertilizer is determined. If an aliquot of the dissolved $^{32}$P labelled fertilizer containing 10 mg P gives an activity of 4000 dpm then the following relationship can be written:

$$4000 \text{ dpm} \quad \text{------} \quad 10 \text{ mg P in the fertilizer}$$

$$800 \text{ dpm} \quad \text{------} \quad X \text{ mg P in the plant}$$
and \( X = \frac{800 \times 10}{4000} = 2 \text{ mg P} \) in the plant came from the fertilizer.

The next step is to calculate the \( P_{dff} \) by relating the amount of P in the plant which came from the fertilizer (2 mg P) to the total amount of P in the plant sample (8 mg P). Thus 2 mg out of 8 mg was derived from the fertilizer and:

\[
P_{dff} = \frac{2}{8} = \frac{1}{4} = 0.25
\]
or \( \% P_{dff} = 25 \%

From the above example, by combining the calculations made before it follows that:

\[
P_{dff} = \frac{800 \times 10}{4000} = \frac{800 \times 10}{4000 \times 8}
\]

\[
P_{dff} = \frac{800}{8} \times \frac{10}{4000}
\]

Since by definition:

\[
\text{S.A. plant} = \frac{800 \text{ dpm}}{8 \text{ mg P}} = 100 \text{ dpm/mg P}
\]

\[
\text{S.A. fertilizer} = \frac{4000 \text{ dpm}}{10 \text{ mg P}} = 400 \text{ dpm/mg P}
\]

Therefore substituting the S.A. of plant and fertilizer in the previous equation the following general equation may be written:

\[
P_{dff} = \text{S.A. plant} \times \frac{1}{\text{S.A. fertilizer}}
\]
or

\[
P_{dff} = \frac{\text{S.A. plant}}{\text{S.A. fertilizer}}
\]

Thus in the present example, \( P_{dff} = \frac{100}{400} = 0.25 \) or \( \%P_{dff} = 25\% \).
Exercise 4:

In a greenhouse experiment, 20 ppm P as $^{32}$P labelled single superphosphate were applied to pots containing 2 kg soil with barley as test crop. After 2 months, the plant material was harvested and analysed for $^{32}$P activity and total P content. The $^{32}$P labelled single superphosphate (standard) used in this experiment was analysed in the same way.

Results:

Plant sample: 5 g dry matter yield

0.2 % total P

An aliquot containing 2 mg P gave 250 cpm $^{32}$P by Cerenkov counting. Since the counting efficiency was 50% the activity of the plant sample was 500 dpm $^{32}$P.

Thus the S.A. plant = $\frac{500 \text{ dpm} \times 32P}{2 \text{ mg P}}$ or 250 dpm $^{32}$P/mg P.

Fertilizer: An aliquot containing 10 mg P counted by Cerenkov gave 6250 cpm $^{32}$P. Considering also 50% counting efficiency the activity of the fertilizer sample was 12500 dpm $^{32}$P.

Therefore S.A. fertilizer = $\frac{12500 \text{ dpm} \times 32P}{10 \text{ mg P}}$ or 1250 dpm $^{32}$P/mg P

Questions:

1. What was the fraction of P in the plant which was derived from the fertilizer or % Pdff?
2. What was the fraction of P in the plant which was derived from the soil?
3. What was the total P yield of the crop?
4. What was the fertilizer P yield of the crop?
5. What was the fertilizer P utilization or recovery by the crop?

Calculations:

1. % P derived from the fertilizer:

$$\% \text{ Pdff} = \frac{\text{S.A. plant}}{\text{S.A. fertilizer}} \times 100$$

$$= \frac{250}{1250} \times 100$$

$$= 20\%$$
2. % P derived from the soil:

\[ \text{% Pdfs} = 100 - \text{% PdFP} \]
\[ = 100 - 20 \]
\[ = 80\% \]

3. The P yield of the crop:

\[ \text{P yield} = 5 \text{ (g)} \times \frac{0.2}{100} = 0.01 \text{ g P/pot} \]
\[ = 5000 \text{ (mg)} \times \frac{0.2}{100} = 10 \text{ mg P/pot} \]

4. The fertilizer P yield of the crop:

\[ \text{Fertilizer P yield} = 10 \times \frac{20}{100} \]
\[ = 2 \text{ mg P/pot} \]

5. Fertilizer P utilization or recovery by the crop: Since 20 ppm P or 20 mg P/kg soil were applied to each pot containing 2 kg soil, thus the P rate was 40 mg P/pot and

\[ \% \text{ Fertilizer P utilization} = \frac{2}{40} \times 100 = 5\% \]

Exercise 5:

In a rice field, 20 kg P/ha as \[^{32}\text{P}\]-labelled single superphosphate were applied at transplanting time. After about six weeks, plants samples were harvested from these treated plots. The dry matter yield amounted to 2500 kg/ha with a total P content of 0.30 %.

A representative plant dry matter sample was ashed and extracted with a known amount of 2N HCl. An aliquot of this extract containing 0.2 mg P was counted by Cerenkov and was found to give a count rate of 320 cpm. At the same time an aliquot of an acid extract of the \[^{32}\text{P}\]-labelled superphosphate containing 0.3 mg P was counted and was found to have a count rate of 3450 cpm.

The counting efficiency was found to be 40 %.

Question:

What was the fertilizer P utilization?
Calculations:

1. % Pdff is calculated as follows:

   Plant sample activity = \( \frac{320}{0.4} \) = 800 dpm

   S.A. plant sample = \( \frac{800}{0.2} \) = 4000 dpm/mg P

   Fertilizer activity = \( \frac{3450}{0.4} \) = 8625 dpm

   S.A. fertilizer = \( \frac{8625}{0.3} \) = 28750 dpm/mg P

   \( \% \text{ Pdff} = \frac{4000}{28750} \times 100 = 13.9 \%

2. The P yield

   P yield = 2500 \times \frac{0.30}{100} = 0.75 \text{ kg P/ha}

3. The fertilizer P yield:

   Fertilizer P yield = 7.5 \times \frac{13.9}{100} = 1.04 \text{ kg P/ha}

4. The fertilizer P utilization:

   \( \% \text{ Fertilizer P utilization} = \frac{1.04}{20} \times 100 = 5.2\% \)

Measurements needed for experiments with \( ^{32}P \) or \( ^{33}P \):

The following basic primary data need to be recorded for field and greenhouse experiments with labelled P fertilizers:

1. Dry matter (D.M.) yield
2. Total nutrient content (% P in dry matter) – Analysed by conventional chemical method
3. Plant specific activity (S.A. plant)
4. Fertilizer specific activity (S.A. fertilizer)
   For the points 3 and 4, two independent measurements are required in each aliquot, i.e. the determination of the \( ^{32}P \) activity by Cerenkov counting and the content of the element i.e. amount of P.
5. Radioisotopically-labelled fertilizer(s) used and the rate(s) of application
Calculations for experiments with $^{32}\text{P}$ and/or $^{33}\text{P}$

The following calculations need to be made:

1. The S.A. of plant and fertilizer

$$\text{S.A. fertilizer}$$

2. \(\%\text{Pdff} = \frac{\text{S.A. plant}}{\text{S.A. fertilizer}} \times 100\) \hfill (12)

3. Dry matter yield per unit area:

$$\text{DM yield (kg/ha)} = \frac{\text{FW (kg)}}{\text{area harvested (m}^2\text{)}} \times \frac{10,000 (m^2/ha)}{\text{SFW (kg)}}$$ \hfill (13)

4. \(\text{P yield (kg/ha)} = \text{DM yield (kg/ha)} \times \frac{\%\text{P}}{100}\) \hfill (14)

5. Fertilizer P yield (kg/ha) = P yield (kg/ha) \times \frac{\%\text{Pdff}}{100} \hfill (15)

6. \(\%\text{fertilizer P utilization} = \frac{\text{Fertilizer P yield}}{\text{Rate of P application}} \times 100\) \hfill (16)

ROOT ACTIVITY STUDIES USING ISOTOPE TECHNIQUES

Introduction

Root studies are becoming increasingly important component of crop improvement and selection programmes. The roots are responsible for plant water and nutrient uptake. Plant and microbes can also form associations at the root level, which may significantly affect crop productivity in different ways. There is a continuous development of new methods for studying root systems. The suitability of one or another method depends basically on the objective(s) of the study and the available resources. Most classical methods (visual observations and/or physical separation of roots) are aiming at determining rooting pattern of crops but do not provide information on root activity, growth and physiological responses to environmental factors. Isotope techniques are unique tools to provide information to many of these. For example they offer a quick and reliable means for determining the distribution pattern of active roots.
Isotope tracer techniques for root activity studies

The tracer methodology consists basically of injecting a suitable isotope either to the soil or to the plant. Two approaches have been adopted in the development of these isotope tracer techniques, as follows:

a) An isotope such as $^{32}\text{P}$ or $^{86}\text{Rb}$ is injected into the plant stem and the pattern of root distribution is determined by taking soil-root cores, and measuring the radioactivity in them.

b) A $^{32}\text{P}$-labelled phosphate solution is injected into the soil at the various positions (distances and depths) and by measuring the radioactivity in the plant samples the root activity (patterns of root activity) at the various positions is tested.

Both isotope tracer techniques have been applied extensively to field crops. Part of this work has been done by the FAO/IAEA programme. A soil injection technique for determining the root activity distribution of various tree crops of economic importance to developing countries was developed within the framework of a Co-ordinated Research Programme of the Joint FAO/IAEA Division. The IAEA Laboratory at Seibersdorf was instrumental in the improvement of the technique by working out the injection and sampling aspects (IAEA, 1975).

Definitions

Root activity: Relative term used to compare various parts of a root system. It is expressed in function of the amounts of nutrients taken up from a common source of nutrient supply.

Root activity ratio: The relationship of the amount of nutrient taken up by two different parts of a root system. The activity ratio may change with time and space.

Root activity distribution pattern: It is the root activity ratio of any part of the root system related to that of an arbitrary standard location.
Applications

The injection technique for root activity studies may be used for the following applications:

To study the effect of land preparation methods on root activity (tillage/plowing techniques for seedbed preparation and erosion control).

To study the effect of cultivation and other methods for weed control.

Evaluating methods of fertilizer placement/timing for orchard and plantation trees as well as wide-row planted annual crops.

Genotypic differences in response to environmental stresses, i.e. soybean cultivars response to moisture stress.

Definition of crop rotation systems, i.e. combination of crops with different root depth or compatibility of nitrogen fertilization of crops.

Root activity patterns of tree crops

Quantitative measurement of fertilizer practices using isotope techniques cannot be readily applied to tree crops. The quantities of labelled fertilizer required are much larger than those for annual crops and therefore, high costs are involved in field experimentation of tree crop plantations. Moreover it is not practical to harvest and sample whole trees. The approach adopted has been to reduce costs through a careful selection of a small number of treatments and replications. More recently further improvement has also been achieved by reducing the amount of labelled fertilizer required to label a tree through the use of advanced analytical equipment for isotopic analysis. This has furthermore been possible by developing and applying a soil injection technique for determining the root activity distribution of various tree crops of economic importance to developing countries (IAEA, 1975).

Rationale

Application of fertilizer in close proximity to the zone of highest root activity and at a time when the roots are most active can be expected to result in a higher fertilizer nutrient uptake by the crop. Information on the distribution pattern of root activity are therefore an essential prerequisite for the formulation of sound fertilizer practices for tree crop plantations.
Injection techniques:

A solution containing a suitable isotope readily translocated and equilibrated with the pool of nutrient in the plant is being used. The measurement can be done by using single labelling: $^{32}\text{P}$, $^{86}\text{Rb}$, $^{15}\text{N}$ or double labelling: $^{32}\text{P}$ and $^{33}\text{P}$ (two different isotopes of the same element).

Injection procedure:

Single trees are used as experimental units. Particular care must be taken to ensure the selection of trees with uniform vegetative characteristics (girth, height, foliage, fruiting capacity).

Each tree is treated with a given total $^{32}\text{P}$ activity distributed in equal aliquots of approximately 5ml containing 1000 ppm P solution. These are inserted into 15-20 holes in a ring around the tree at a specific depth and distance from the tree to be tested.

A wide variety of equipment and devices can be used for preparing the holes and injecting the solution in the holes (injection points).

Plant sampling and analysis

i) Sampling is the main difference between annual (whole plants) and perennial (leaves) crops. In the case of tree crops, two types of leaf sampling may be considered:

Bulk sample: Representative sample obtained by systematic sampling of leaves from the entire tree, i.e.: counting the leaves and taking every fourth or tenth leaf (depending on the type and size of tree) from every twig and branch.

Standard sample: Representative sample of leaves from a well-defined morphological position. The sample recommended for foliar diagnosis is also used for this purpose.

ii) Sampling time interval: Sufficient time (a few weeks) after the injection should elapse so that the nutrient can be translocated and redistributed uniformly throughout the entire canopy. This is a condition for the method to be valid.
iii) **Sampling size**: It is a function of the activity of the sample. Since the counting rates are low, usually 5-10 g of oven-dry leaf material is used for analysis.

iv) **Analysis**: The isotope ($^{32}P/^{33}P$) activity in ashed leaves samples in HCl extract is determined by counting using a liquid scintillation counter.

v) **Interpretation**: For each experiment, the count rates are corrected for decay to a pre-set time and the results are expressed in dpm/g dry matter.

The activity of the sample is a measure of the root activity. A qualitative comparison of the root activity at different locations (injection treatments) can be made.

**Sources of error**

i) **Soil (spatial) variability across the plantation.**

ii) **Plant variability (genetic origin).**

iii) **Sampling factors** (type, size, time, etc.). They are the main source of experimental error. It can be reduced to some extent by increasing the number of subsamples, particularly in the case of "standard" sampling. In practice, bulk sampling results in a significant reduction of the sampling variation.

iv) **Injection factors**. Unequal probability of contact between roots and the applied isotope. This may occur if the number of injection points are too small.

v) **Eccentricity factor**. Unequal probability of contact between roots and the applied isotope at different distances tested. This is the case when the number of injection points per unit length of circumference for distances close to the trunk is greater than that for circumferences further out from the trunk.
Exercise 6: Single labelling technique

This exercise is to illustrate the single labelling technique. A $^{32}\text{P}$ labelled phosphate solution was injected in regular patterns into the soil. The $^{32}\text{P}$ activity in the leaves of the tree is a measure of the corresponding root activity of the location where the $^{32}\text{P}$ was injected into the soil.

Objective

To determine the area of highest root activity in a 3-year old oil palm plantation.

Application treatments

1) Inside a clean weeded ring
2) At the crown's diameter
3) Outside the crown's diameter

1 palm = 1 experimental unit
3 treatments x 6 replications = 18 palms

Experimental procedure

Injection: About 320 µCi $^{32}\text{P}$/palm as 16 injections of 5 ml 1000 ppm $\text{P}$ solution containing 20 µCi $^{32}\text{P}$ each were injected in rings around the base of the palm into the top 5 cm of the soil.

A bulk sample of leaves was taken by systematic sampling of the entire crown. Each branch was sampled systematically by taking every tenth leaf. Midribs were removed and only centre 10 cm of each leaflet taken as subsample. After quartering, the final subsample was weighed, oven-dried at 70°C, weighed again for dry matter determination, ashed and dissolved in 50 ml 2N HC1. The activity was determined by Cerenkov counting in a liquid scintillation counter and the final results expressed in dpm $^{32}\text{P}$/g dry matter are shown in Table 3.
Table 3: Results of Cerenkov counting of leaves from palm trees after application of $^{32}$P into the soil by various treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>312</td>
<td>110</td>
<td>570</td>
<td>480</td>
<td>410</td>
<td>220</td>
</tr>
<tr>
<td>(2)</td>
<td>730</td>
<td>942</td>
<td>751</td>
<td>1160</td>
<td>1012</td>
<td>852</td>
</tr>
<tr>
<td>(3)</td>
<td>120</td>
<td>90</td>
<td>260</td>
<td>20</td>
<td>213</td>
<td>160</td>
</tr>
</tbody>
</table>

Conclusions:

i) The highest activity was found to be at the crown diameter

ii) Despite the high standard deviation, the differences among treatments were highly significant.

Exercise 7: Double labelling technique

In this example the double labelling technique with $^{32}$P and $^{33}$P will be illustrated.

Trees are individuals and despite the fact that all the trees in a plantation block are of the same genetic origin, age, etc, the variability in nutrient uptake among individual trees may be large. This cannot be solved by improving sampling methods. It could be improved by drastically increasing the number of replications for each treatment but this is usually too costly. Therefore, benefiting of the availability of two radioisotopes for phosphorus, a double labelling technique was developed.

This technique consists of injecting solutions labelled with $^{32}$P in one location and $^{33}$P in another location around the same tree. The difference between areas of root activity is measured in terms of the ratio of uptake from $^{32}$P and $^{33}$P.
Objective

To determine the root activity of oil-palm trees in six different locations (combinations of distance and depth).

Experimental procedure

Injection: 16 injections of 5 ml 1000 ppm P\(^{32}\)P-labelled solution were given in rings according to the experimental treatments. Similarly 16 injections of 5 ml 1000 ppm P\(^{33}\)P-labelled solution were applied in the same "standard" location, as illustrated below:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(32^P) injections</th>
<th>(33^P) injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Distance (m)})</td>
<td>(\text{Depth (cm)})</td>
<td>(\text{Distance (m)})</td>
</tr>
<tr>
<td>(1)</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>(2)</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>(3)</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>(4)</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>(5)</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>(6)</td>
<td>3</td>
<td>20</td>
</tr>
</tbody>
</table>

For this experiment 6 treatments x 4 replications = 24 palms were used.

Sampling subsampling and analyses were done as indicated for exercise 6. Counting of dual labelled (\(^{32}\)P and \(^{33}\)P) samples were done after sample preparation.
### Table 4: Activities in samples from palm trees after various $^{32}$P and $^{33}$P treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Isotope</th>
<th>Replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>(1)</td>
<td>$^{32}$P</td>
<td>1050</td>
</tr>
<tr>
<td></td>
<td>$^{33}$P</td>
<td>350</td>
</tr>
<tr>
<td>(2)</td>
<td>$^{32}$P</td>
<td>3517</td>
</tr>
<tr>
<td></td>
<td>$^{33}$P</td>
<td>360</td>
</tr>
<tr>
<td>(3)</td>
<td>$^{32}$P</td>
<td>512</td>
</tr>
<tr>
<td></td>
<td>$^{33}$P</td>
<td>334</td>
</tr>
<tr>
<td>(4)</td>
<td>$^{32}$P</td>
<td>412</td>
</tr>
<tr>
<td></td>
<td>$^{33}$P</td>
<td>343</td>
</tr>
<tr>
<td>(5)</td>
<td>$^{32}$P</td>
<td>1103</td>
</tr>
<tr>
<td></td>
<td>$^{33}$P</td>
<td>370</td>
</tr>
<tr>
<td>(6)</td>
<td>$^{32}$P</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$^{33}$P</td>
<td>358</td>
</tr>
</tbody>
</table>

**Calculations**

In order to compare the root activity in the 6 locations, it was necessary to determine the ratio corresponding to the actual amounts of phosphorus taken up. For this purpose the $^{33}$P counts had to be transformed into equivalent $^{32}$P counts. The following calculations were made:

The conversion factor to transform $^{33}$P counts into equivalent $^{32}$P counts was determined. Using the data of treatment 1 (standard) where the $^{32}$P and $^{33}$P injections were given in the same location. The activity of $^{32}$P represent the same amount of phosphorus (carrier phosphate) taken up as the activity of $^{33}$P. Thus in Treatment 1, replication I 1050 dpm $^{32}$P represent the same uptake of carrier phosphate as 350 dpm $^{33}$P and the corresponding ratio is $\frac{^{32}}{^{33}}P = \frac{1050}{350} = 3$
In the same way the \( ^{32}P/^{33}P \) ratios for the other replications of treatment 1 are calculated.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1050/350 = 3.00</td>
</tr>
<tr>
<td>II</td>
<td>830/290 = 2.86</td>
</tr>
<tr>
<td>III</td>
<td>417/130 = 3.20</td>
</tr>
<tr>
<td>IV</td>
<td>610/215 = 2.84</td>
</tr>
</tbody>
</table>

Mean 2.98 (Average conversion factor)

The transformation of \( ^{33}P \) counts into equivalent \( ^{32}P \) counts and the calculation of activity ratios for each treatment are shown in Table 5.

Table 5: Transformation of \( ^{33}P \) counts into equivalent \( ^{32}P \) counts by multiplying the \( ^{33}P \) counts by 2.98 and calculation of the ratio for each treatment

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>(dpm/g DM)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>( ^{32}P )</td>
<td>1050</td>
<td>830</td>
<td>417</td>
<td>610</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{33}P \rightarrow ^{32}P )</td>
<td>1050</td>
<td>830</td>
<td>417</td>
<td>610</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>( ^{32}P )</td>
<td>3517</td>
<td>3210</td>
<td>1509</td>
<td>2012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{33}P \rightarrow ^{32}P )</td>
<td>1073</td>
<td>983</td>
<td>417</td>
<td>581</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>3.3</td>
<td>3.3</td>
<td>3.6</td>
<td>3.5</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>( ^{32}P )</td>
<td>512</td>
<td>780</td>
<td>309</td>
<td>121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{33}P \rightarrow ^{32}P )</td>
<td>995</td>
<td>1550</td>
<td>566</td>
<td>298</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>0.51</td>
<td>0.50</td>
<td>0.55</td>
<td>0.41</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>( ^{32}P )</td>
<td>412</td>
<td>815</td>
<td>1025</td>
<td>517</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{33}P \rightarrow ^{32}P )</td>
<td>1022</td>
<td>1967</td>
<td>2092</td>
<td>1228</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>0.40</td>
<td>0.41</td>
<td>0.49</td>
<td>0.42</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>( ^{32}P )</td>
<td>1103</td>
<td>938</td>
<td>1269</td>
<td>713</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{33}P \rightarrow ^{32}P )</td>
<td>1103</td>
<td>900</td>
<td>1401</td>
<td>775</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>1.0</td>
<td>1.0</td>
<td>0.9</td>
<td>0.9</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>( ^{32}P )</td>
<td>0</td>
<td>110</td>
<td>50</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ^{33}P \rightarrow ^{32}P )</td>
<td>1067</td>
<td>1162</td>
<td>2247</td>
<td>1028</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>0</td>
<td>0.02</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>
Summary of results

<table>
<thead>
<tr>
<th>DISTANCE</th>
<th>1.0m.</th>
<th>2.0m.</th>
<th>3.0m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10cm</td>
<td>1</td>
<td>3.4</td>
<td>0.5</td>
</tr>
<tr>
<td>DEPTH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20cm</td>
<td>0.4</td>
<td>1.0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Conclusions

i) The zone of highest root activities is located 2.0m. from the base of the tree and at 10cm. depth.

ii) The root activity at 10cm depth is always higher than at 20cm depth.

iii) Variability of ratios is less than that of activities (dpm/g dry matter).

QUANTIFICATION OF FERTILIZER UPTAKE WITHOUT PLANT-FERTILIZER INTERATIONS

Fertilizer management practices such as sources, timing, placement, etc. may be studied in presence and absence of the effects of the fertilizer treatments on plant development, root distribution and crop yield. The use of a special design with isotope techniques allows the study of the fertilizer uptake without plant-fertilizer interaction. (Single-treatment fertility experiments, Broeshart, 1974; Fried et al., 1975).

Example 1:

Timing (T) of fertilizer N application (90 kg N/ha applied to maize at 3 different times).
Design I: Fertilizer treatments with interaction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T&lt;sub&gt;1&lt;/sub&gt;</th>
<th>T&lt;sub&gt;2&lt;/sub&gt;</th>
<th>T&lt;sub&gt;3&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>90*</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>90*</td>
</tr>
</tbody>
</table>

Design II: Fertilizer treatments without interaction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T&lt;sub&gt;1&lt;/sub&gt;</th>
<th>T&lt;sub&gt;2&lt;/sub&gt;</th>
<th>T&lt;sub&gt;3&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>la</td>
<td>30*</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>lb</td>
<td>30</td>
<td>30*</td>
<td>30</td>
</tr>
<tr>
<td>lc</td>
<td>30</td>
<td>30</td>
<td>30*</td>
</tr>
</tbody>
</table>

All fertilizer treatments (la, lb, lc) are identical with regard to the total N rate (90 kg N/ha) and 3-split of 30 kg N/ha at all timing treatments. Only the position of the labelled fertilizer changes, thus the effect of timing would be measured in the absence of any plant-fertilizer interaction effect. Therefore by utilizing design II, the partial fertilizer use efficiency of the 30 kg N/ha applied at each timing and the total fertilizer use efficiency of a 3-split application of 90 kg N/ha can be measured without any interaction effect.

Example 2:

Fertilizer N placement. In this experiment 80 kg N/ha were applied to sorghum in 2 different placements.
Design I: Fertilizer treatments with interaction

<table>
<thead>
<tr>
<th>Surface broadcast over plot area (S)</th>
<th>Band at 5 cm depth in a furrow (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80*</td>
</tr>
<tr>
<td>2</td>
<td>--</td>
</tr>
</tbody>
</table>

Design II: Without interaction plant-fertilizer.

<table>
<thead>
<tr>
<th>(S)</th>
<th>(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>40*</td>
</tr>
<tr>
<td>1b</td>
<td>40</td>
</tr>
</tbody>
</table>

Example 3:

Fertilizer nutrient sources.

Nutrient ion uptake from a single fertilizer source

<table>
<thead>
<tr>
<th>la</th>
<th>*NO₃ NH₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>lb</td>
<td>NO₃ *NH₄</td>
</tr>
</tbody>
</table>

Interactions of nutrient uptake

Effect of N on P uptake

<table>
<thead>
<tr>
<th>la</th>
<th>N and P* separated (in different furrows)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lb</td>
<td>N and P* mixed (in the same furrow)</td>
</tr>
</tbody>
</table>

Effect of P on N uptake

<table>
<thead>
<tr>
<th>2a</th>
<th>N* and P separated (in different furrows)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2b</td>
<td>N* and P mixed (in the same furrow)</td>
</tr>
</tbody>
</table>

N* : ammonium - N source
P* : orthophosphate monovalent - P soluble source
When a commercial fertilizer is applied to the soil, the plant will take up nutrient from both sources, the soil and the applied fertilizer. If increasing fertilizer units are added to a given soil, a nutrient uptake response curve can be obtained. This nutrient uptake curve is made up utilizing a version of the Law of Diminishing Returns, i.e. continuous curvilinear functions (quadratic, square root or logarithmic models), in which equal additional increments of fertilizer result in steadily smaller responses. Relative nutrient uptake (nutrient uptake expressed as percentage of maximum uptake) rather than the amount of nutrient taken up is usually plotted in the ordinate. At some point maximum uptake will be obtained after which no more of the fertilizer nutrient applied will be taken up. At a higher level of fertilizer application there may even be a decrease in uptake of the nutrient. The curve is made up of both sources, i.e. uptake from the soil and uptake from the fertilizer. There is no way of knowing from the curve how much is taken up from the soil and how much from the fertilizer (Broeshart, 1974; Fried, 1978b and Vose, 1980).

The concept of available amounts of a nutrient

When comparing different nutrient sources, the term "available amount of a nutrient" has first to be defined. Only the plant can judge what is available since no chemical extraction can determine what is available to a plant. However, if the plant is used to measure which source of nitrogen or phosphorus is available and to which extent, one has to be able to discriminate between the sources, which is conveniently done by labelling one of the sources with an appropriate isotope.

Also when comparing nutrient sources one wants to know how much available nitrogen or phosphorus in a given source the plant sees in comparison with well-known fertilizers. Thus, if a rock phosphate is applied we want to know what is the available amount of phosphorus in the rock phosphate in terms of equivalent units of superphosphate or in other words how many kg of rock phosphate supply the same amount of phosphorus to a crop as one kg of superphosphate. However, since the soil is a source of nutrients, the first question which may be asked is: What is the available amount of phosphorus in the soil in terms of equivalent units of superphosphate or what is the available amount of nitrogen in the soil in terms of equivalents units of ammonium sulphate? Thus by utilizing a labelled fertilizer source it is possible to
determine the plant available amount of nutrient in the soil and this amount
is expressed relative to the amount available in the fertilizer source.

For example, if 100 kg N/ha as $^{15}$N labelled ammonium sulphate is added
to the soil and plant analysis gave $\%\text{Ndfs} = 50\%$. Thus $\%\text{Ndff} = 50\%$. It could
then be said that the soil had the same amount of available nitrogen as the
100 kg N/ha added as fertilizer or in other words the soil had an available
amount of 100 kg N/ha in terms of ammonium sulphate equivalent units.

What will be the consequence when 200 kg N/ha are added to the same
soil? Since the soil has an amount of available nitrogen equivalent to 100 kg
N/ha and the fertilizer 200 kg N/ha, the proportion taken up by the plant from
the soil will be one third and two thirds from the fertilizer.

What will be the consequence when 300 kg N/ha are added? The proportion
taken up from the soil will be one fourth and three fourths from the
fertilizer.

Thus the following assumption may be formulated: "When a plant is
confronted with two or more sources of a nutrient element, the nutrient uptake
from each of these sources is proportional to the amounts available in each
source" (Fried and Dean, 1952; Fried, 1964; Broeshart, 1974; Fried, 1978b and
Vose, 1980).

The above relationships which are also called fractional utilization
ratios can be expressed in the form of an equation. Thus, in a situation
where soil and fertilizer are the only sources of nutrient available to a
plant, the equation is as follows:

$$\frac{\text{Total nutr. in plant}}{\text{Total nutr. supply}} = \frac{\text{Fert. nutr. in plant}}{\text{Fert. nutr. supply}} = \frac{\text{Soil nutr. in plant}}{\text{Soil nutr. supply}}$$

(17)

or:

$$\frac{\text{Total nutr. in plant}}{\text{Total nutr. supply}} = \frac{\text{Fert. nutr. in plant} + \text{Soil nutr. in plant}}{\text{Fert. nutr. supply} + \text{Soil nutr. supply}}$$

(18)

For nitrogen as an example,

$$\frac{\text{Total N in plant}}{\text{Total N supply}} = \frac{\text{Fertilizer N in plant}}{\text{Fertilizer N supply}} = \frac{\text{Soil N in plant}}{\text{Soil N supply}}$$

(19)

$$\frac{\text{Total N in plant}}{\text{Total N supply}} = \frac{\text{Fertilizer N in plant} + \text{Soil N in plant}}{\text{Fertilizer N supply} + \text{Soil N supply}}$$

(20)
The fertilizer N supply is equivalent to the rate of N applied as fertilizer. The soil N supply corresponds to the amount of soil N in terms of fertilizer units which is available to the plant during the growth period. Both soil and fertilizer N, as well as total N supply are expressed in the same way, i.e. as equivalent units of applied fertilizer, for example, in kg N/ha of equivalent units of ammonium sulphate, if this was the fertilizer used.

The following exercises are given to illustrate the determination of the available amount of a nutrient in the soil.

Exercise 8 (Greenhouse experiment)

A $^{32}$P labelled superphosphate was applied at a rate of 40 mg P/pot, each containing 1 kg air-dried soil. Barley was used as test crop. After 5 weeks the plants were harvested and analysed for total P and $^{32}$P activity. From the results of the specific activity of plants and fertilizer, it is found that $\%P_{dfs} = 20\%$. What was the available amount of soil P as measured in superphosphate units?

$\%P_{dfs} = 100 - 20 = 80\%$

The following fractional utilization ratio may be written:

\[
\frac{20\%}{40 \text{ mg P as super}} = \frac{80\%}{X}
\]

\[
X = \frac{80\% \times 40 \text{ mg P}}{20\%}
\]

\[
X = 160 \text{ mg P as superphosphate}
\]

Therefore it may be concluded that this soil has an available amount of 160 mg P/kg soil as superphosphate equivalent units.

Exercise 9 (Greenhouse experiment)

To determine the available amount of N in a soil, $^{15}$N labelled urea (1 % $^{15}$N a.e.) was applied at a rate of 80 mg N/pot, each containing 1 kg air-dried soil. Barley, was used as test crop but oat, ryegrass or any other fast-growing plant could have been used.
From the plant analysis it was found that a plant sample had 0.25% $^{15}$N atom excess.

\[
\% \text{Ndff} = \frac{0.25}{1.00} \times 100 = 25 \%
\]

Therefore \(\% \text{Ndfs} = 100 - 25 = 75\%\).

Thus the fractional utilization ratio is as follows:

\[
\begin{align*}
\frac{25\%}{80 \text{ mg N as urea}} &= \frac{75\%}{X} \\
X &= \frac{75\% \times 80 \text{ mg N}}{25\%} \\
X &= 240 \text{ mg N as urea}
\end{align*}
\]

Therefore, this soil has an available amount of 240 mg N/kg soil as urea equivalent units.

Exercise 10 (Field experiment)

100 kg N/ha as $^{15}$N labelled ammonium sulphate were applied to a sorghum crop. After harvest, the plant and fertilizer samples were analysed for total N and for nitrogen isotopic ratio. It was found that the sorghum crop had 120 kg N/ha total N yield and 40 kg N/ha fertilizer N yield.

Questions:

1. What was the available amount of N in the soil as ammonium sulphate equivalent units?

2. What was the Ndff in the plant sample?

Calculations:

\[
\begin{align*}
120 \text{ kg N/ha total} & \quad \text{(total N yield)} \\
40 \text{ kg N/ha from fertilizer} & \quad \text{(fert. N yield)} \\
80 \text{ kg N/ha from soil} & \quad \text{(soil N yield), by difference}
\end{align*}
\]
Then the following relationship may be written:

\[
\frac{40 \text{ kg N/ha (fert.)}}{100 \text{ kg N/ha as ammonium sulphate}} = \frac{80 \text{ kg N/ha (soil)}}{X}
\]

Therefore the available amount of N in the soil is 200 kg N/ha as ammonium sulphate equivalent units.

\[\text{Ndff} = \frac{40}{120} = \frac{1}{3}\]

Thus \[\text{Ndfs} = 1 - \frac{1}{3} = \frac{2}{3}\]

When the calculation is made with the Ndff values, the same result must be obtained. The fractional utilization ratio is as follows:

\[
\frac{1/3}{100} = \frac{2/3}{X}
\]

Where \(X\) = available amount of N in the soil or 200 kg N/ha as ammonium sulphate equivalent units.

It is obvious that the same result must be obtained since the first relationship can be also written as follows:

\[
\frac{1}{3} \times 120 = \frac{2}{3} \times 120
\]

The available amount of nutrient in the soil or the soil nutrient supply, measured in equivalent units of the fertilizer standard has been referred to as the "A-value" (Fried and Dean, 1954).

In a simplest case, when a plant is confronted with only two nutrient sources, i.e. the native soil nutrient pool (ndfs) and the labelled fertilizer nutrient (ndff) supplied at a given rate.

Thus:

\[\% \text{ ndfs} + \% \text{ ndff} = 100\]  \hspace{1cm} (21)

and:

\[\% \text{ ndfs} = 100 - \% \text{ ndff}\]  \hspace{1cm} (22)
As mentioned before, since the calculation is based on the fractional relationship it follows that:

\[
\frac{\text{Fert. nutrient in plant}}{\text{Available amount of fert. nut.}} = \frac{\text{Soil nutrient in plant}}{\text{Available amount of soil nutrient}} \tag{23}
\]

Where:

- Fertilizer and soil nutrient in the plant are the respective proportions of nutrient taken up from each source. The percentage nutrient in the plant derived from the fertilizer is experimentally determined using isotopically labelled fertilizer.

- The available amount of fertilizer nutrient (\(B\)) is the rate of nutrient application as fertilizer standard. It should be noted that as far the plant is concerned, one fertilizer unit is the same as any other fertilizer unit. Thus for instance two fertilizer units contain twice as much available nutrient as one fertilizer unit.

- The available amount of soil nutrient or the A value of the soil for the particular nutrient under study, is expressed in equivalent units of the applied fertilizer.

Therefore the above equation (23) may be written as follows:

\[
\frac{\%\text{ndff}}{B} = \frac{100 - \%\text{ndff}}{A} \tag{24}
\]

And solving for \(A\):

\[
A = \frac{100 - \%\text{ndff}}{\%\text{ndff}} \times B \tag{25}
\]

In determining A values, it is important to note the following:

1) Since the available amount of nutrient in the soil is an inherent property of the soil, it will be constant for any one set of experimental conditions.

2) The A value is a yield-independent parameter. It is only necessary to determine the respective proportions absorbed from each source, so as to determine the A value of the soil. No yield data need to be recorded. The absolute amounts of nutrient taken up from either source do not appear in the equation.
3) The A value for a particular soil remains constant even at different rates of application of the same fertilizer standard. In other words, the available amount of nutrient in the soil is independent of the rate of fertilizer applied. Thus, in soil fertility studies, it is sufficient to use only one rate of application to assess the nutrient supply of a soil and make relative comparisons of fertilizer treatments (Aleksic et al., 1968; Broeshart, 1974).

4) Any change in the set of experimental conditions will affect the magnitude of the A value of the soil. For instance any change in the application (nature, source, placement, timing, etc) of the labelled fertilizer standard or in the growth conditions of the crop. Also changes in harvesting times are important, since the plant samples the nutrient isotopic composition of the soil from the seeding time until harvesting time. These changes of the A value of a soil with time can be easily observed in a time course study of nutrient uptake using labelled fertilizers (Rennie, 1969; Smith and Legg, 1971; Broeshart, 1974; Zapata et al., 1987).

5) The determination of the A value of the soil has a number of practical applications, such as the quantitative evaluation of fertilizer practices, in particular fertilizer sources, and the design of further isotope-aided experiments (Rennie, 1969; Broeshart, 1974; Fried, 1978b; IAEA, 1983).

6) Extensive research work using A values has been done for most plant nutrients, both macro- and micronutrients (Fried, 1954; IAEA, 1976; IAEA, 1980; Vose, 1980; IAEA, 1981; Wagner and Zapata, 1982).

**Relation between soil and fertilizer nutrient uptake**

If the proportion of a nutrient taken up from each source and the total nutrient uptake is known, the actual amount taken up from each source can be calculated. Also it is assumed that when a plant is confronted with two sources of a nutrient it will take up nutrient from each source in direct proportion to the available amounts of that nutrient in each source. This is graphically represented in Fig. (1) by a theoretical nitrogen uptake curve of a crop where the nitrogen yield has been plotted as a function of the nitrogen supply. Abundant evidence obtained from experiments with labelled fertilizers has confirmed the assumption made for Fig. 1. The curves for soil N = 100, 200, 300 and 400 represent the amounts taken up from the soil by the crop in proportion to the different ratios of soil and fertilizer N.
The nutrient uptake from the soil is a function of the relative available amounts of nutrient from the soil (soil nutrient supply) and fertilizer (fertilizer nutrient supply).

It is evident that the nutrient uptake from the soil will change each time the rate of fertilizer application (fertilizer nutrient supply) changes even though the A value is constant. The change in nutrient uptake from soil due to fertilizer application has been described as "priming". Furthermore it is known that the actual amount of nutrient taken up from the soil in fertilized treatments may be higher, the same or lower than the amount taken up by a control treatment (Westerman and Kurtz, 1973; Fried and Broeshart, 1974).

The following series of exercises will illustrate the concepts mentioned above:

<table>
<thead>
<tr>
<th>Example 1</th>
<th>Example 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil N supply</td>
<td>100</td>
</tr>
<tr>
<td>Fert N supply</td>
<td>0</td>
</tr>
<tr>
<td>Total N supply</td>
<td>100</td>
</tr>
<tr>
<td>Total N uptake</td>
<td>20</td>
</tr>
<tr>
<td>Uptake from fertilizer</td>
<td>0</td>
</tr>
<tr>
<td>Uptake from soil</td>
<td>20</td>
</tr>
</tbody>
</table>
From example 1: (Fig. 1)

The N uptake from the soil increased from 20 kg N/ha in the control treatment to 32 kg N/ha in the fertilized treatment due to the fertilizer application. This is called "positive priming". Generally this is only the case when there is a pronounced increase in uptake from both soil and fertilizer (N yield response) because the soil has a relative low available amount of N.

From the example 2: (Fig. 1)

The opposite may be observed, where the N uptake from the soil decreased from 98 kg N/ha in the control treatment to 87 kg N/ha in the fertilized treatment as a result of the fertilizer application. This is known as "negative priming". In this case there is no N yield response because the soil has a high available amount of N.

Exercise 11:

Objective: To demonstrate the effect of rate of fertilizer application on the uptake of nutrient from the soil.

A soil has an available amount of 150 kg N/ha as ammonium sulphate equivalent units. The control treatment shows an uptake of 70 kg N/ha. What will be the effect of the application of 100, 200 and 300 kg N/ha as ammonium sulphate on the N uptake from the soil?

<table>
<thead>
<tr>
<th></th>
<th>Control treatment</th>
<th>Fertilized treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Soil N supply</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Fert N supply</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Total N supply</td>
<td>150</td>
<td>250</td>
</tr>
<tr>
<td>Total N uptake</td>
<td>70</td>
<td>135</td>
</tr>
<tr>
<td>Uptake from fertilizer</td>
<td>0 250 x 135=54</td>
<td>200 350 x 168=96</td>
</tr>
<tr>
<td>Uptake from soil</td>
<td>70 250 x 135=81</td>
<td>150 350 x 168=72</td>
</tr>
</tbody>
</table>
The results shown above are graphically represented in Fig. 2. The following may be observed:

Treatment I: Due to the application of 100 kg N/ha the uptake of nutrient from the soil increased over the control treatment. (Positive priming).

Treatment II: When 200 kg N/ha was applied the amount of N taken up from the soil was approximately equal to the amount taken up by the control. (No priming).

Treatment III: When 300 kg N/ha was applied the amount of N taken up from the soil was less than the amount of nutrient taken up by the control treatment. (Negative priming).

Conclusions:


(2) There is no effect of fertilizer N application on soil N supply or in other words the A value of the soil is independent of the rate of fertilizer N applied for any one set of experimental conditions.

(3) The N uptake from soil when fertilizer is added may be higher, the same or lower than the N uptake from the control plot. This can happen even
though the A value is constant. Thus the so-called "priming" effect is only a reflection of the change in N uptake from soil due to the mathematical consequence of adding fertilizer to the system. No mechanistic explanation is necessary for such an observation.

(4) It also follows that to assume that the amount of nutrient taken up from the soil is constant at different rates of fertilizer application is inherently false. (Difference method).

(5) The so-called difference method for estimating fertilizer nutrient uptake as the difference in nutrient uptake between the control and fertilized treatments, is based on the wrong assumption that the amount of N taken up by the control treatment measures the amount of N taken up from the soil in the fertilized treatments. Therefore this method has been found to under-estimate or over-estimate largely the fertilizer nutrient uptake by various crops (Aleksic et al., 1968; Broeshart, 1974; Westerman and Kurtz, 1974; Harmsen and Moraghan, 1988).

(6) A direct and quantitative measurement of fertilizer nutrient uptake can be made by using isotopically labelled fertilizers. Thus, the proportion of nutrient in the plant derived from the fertilizer is experimentally determined and that from the soil is subsequently calculated. The available amount of nutrient in the soil can be quantitatively expressed in terms of fertilizer standard equivalent units. Knowing the proportions of nutrient taken up from each source and the total nutrient uptake by the crop, the actual amounts of nutrient taken up from each source can also be calculated.

QUANTITATIVE EVALUATION OF FERTILIZER PRACTICES

The following nutrient supply from several fertilizer management practices can be quantitatively evaluated using isotope techniques:

1. Method of placement,
2. Timing of application,
3. Chemical and physical nature of sources, including symbiotic nitrogen fixation,
4. Interaction among topics 1, 2 and 3, and of these with cultural practices (irrigation, mulching, tillage, etc.).

For instance one may ask how much is placement method A better than method B. Also when comparing different fertilizer sources, one wants to know how much available phosphorus or nitrogen the plant sees from a given nutrient source
in comparison with well-known fertilizers. Thus, when rock phosphate is applied one wants to know how many kg of rock supply the same available amounts of phosphorus to a crop as one kg superphosphate.

The following series of examples illustrate the quantitative evaluation of fertilizer practices with annual crops. It essentially consists of equating the two comparisons with each other and is based on the principle that the available amount of nutrient in the soil, which is identical for all treatments, is expressed in units of each fertilizer treatment. This enables a direct comparison of fertilizer equivalent units among different treatments.

**Method of fertilizer placement**

A maize field experiment was carried out to compare a band placement to a surface-broadcasted application of 120 kg N/ha as $^{15}$N-labelled ammonium sulphate.

**Results:**

<table>
<thead>
<tr>
<th></th>
<th>Banding</th>
<th>Surface broadcast</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Ndff</td>
<td>65</td>
<td>46</td>
</tr>
<tr>
<td>% Fert. N util.</td>
<td>78</td>
<td>48</td>
</tr>
</tbody>
</table>

The farmer used to apply 120 kg N on the surface. If in the future he decides to apply the fertilizer in a band, what should be the rate of N application in order that the maize crop takes up the same amount of N from banding as previously from surface application?

**Principle:**

The available amount of soil N seen by the maize crop is the same for banding and surface treatments. Therefore we express the available amount of soil N in equivalent banding and surface units, which will enable us to calculate how many kg N given in a band supply the same amount of N to the maize as 120 kg N on the surface.
Banding treatment

%Ndff = 65\% and %Ndfs = 35\%.

From the fractional utilization relationship:

\[
\frac{65}{120} = \frac{35}{X}
\]

Thus \( X \) or the available amount of soil N is 65 kg N/ha in banding equivalent units.

Surface treatment

%Ndff = 46\% and %Ndfs = 54\%

From the fractional utilization relationship:

\[
\frac{46}{120} = \frac{54}{X}
\]

Thus \( X \) or the available amount of soil N is 141 kg N/ha in surface equivalent units.

The experimental soil has an available amount of N equivalent to 65 kg N/ha as banding units or 141 kg N/ha as surface units. In other words, 65 kg N/ha in a band supplies the same amount of N to the maize crop as 141 kg N/ha on the surface.

\[
65 \text{ kg N in a band} = 141 \text{ kg N on the surface}
\]

\[
X = \frac{65}{141} = 0.46 \text{ kg N in a band}
\]

Instead of the application of 120 kg N/ha on the surface, the farmer should apply 0.46 \times 120 = 55 kg N/ha in a band to get the same amount of fertilizer taken up.

**Timing of fertilizer application**

A field experiment was carried out to compare the available amounts of N in various \(^{15}\text{N}\)-labelled urea treatments on winter wheat. One single application of 100 kg N/ha urea in the autumn was compared against a two-
split application of 50 kg N/ha at tillering stage + 50 kg N/ha at heading stage.

Results:

<table>
<thead>
<tr>
<th></th>
<th>Single application</th>
<th>Two-split application</th>
</tr>
</thead>
<tbody>
<tr>
<td>% N def</td>
<td>37</td>
<td>44</td>
</tr>
<tr>
<td>A values</td>
<td>170</td>
<td>127</td>
</tr>
</tbody>
</table>

The two-split application was found to be superior to the single application. By how much is the two-split application better than the single application?

The soil has an available amount of N equivalent 170 kg N/ha as single application units or 127 kg N/ha as two-split application units.

Therefore

\[
170 \text{ kg N in a single application} = 127 \text{ kg N in a two-split application}.
\]

\[
1 \text{ kg N} \quad \text{in a single application} = X
\]

\[
1 \text{ kg N in a single application is equivalent to } X = \frac{127}{170} = 0.75 \text{ kg N in a two-split application}.
\]

Therefore the two-split application is \( \frac{1}{0.75} \) or 33% better than the single application.

**Fertilizer nutrient sources**

**Fertilizer sources which can be labelled (Direct method)**

A) Comparison of N sources i.e.: Two fertilizers N such as urea and ammonium sulphate.

Treatments:

\(^{15}\text{N-labelled urea, and }^{15}\text{N-labelled ammonium sulphate, both}\)

applied a rate of 100 kg N/ha
Results:

Urea treatment:

\[ \text{N yield} = 120 \text{ kg N/ha} \]
\[ \text{Plant sample} = 1.2 \%^{15} \text{N atom excess} \]
\[ \text{Fertilizer Sample} = 2.0 \%^{15} \text{N atom excess} \]

\[ \% \text{ Ndff} = \frac{1.2}{2.0} \times 100 \]
\[ = 60\% \]
\[ \% \text{ Ndfs} = 40\% \]

From the fractional utilization relationship

\[ \frac{60}{100} = \frac{40}{X} \]
\[ X = \text{available amount of soil N or 66.7 kg N/ha in urea equivalent units.} \]

Ammonium sulphate treatment:

\[ \text{N yield} = 105 \text{ kg N/ha} \]
\[ \text{Plant sample} = 0.6 \%^{15} \text{N atom excess} \]
\[ \text{Fertilizer Sample} = 1.2 \%^{15} \text{N atom excess} \]

\[ \% \text{ Ndff} = \frac{0.6}{1.2} \times 100 \]
\[ = 50\% \]
\[ \% \text{ Ndfs} = 50\% \]

From the fractional utilization relationship

\[ \frac{50}{100} = \frac{50}{X} \]
\[ X = \text{available amount of soil N or 100 kg N/ha in ammonium sulphate equivalent units.} \]
Quantitative comparison of urea and ammonium sulphate.

\[66.7 \text{ kg N as urea} = 100 \text{ kg N as ammonium sulphate}\]

\[\frac{\text{X}}{1 \text{ kg N as ammonium sulphate}} = \frac{66.7}{100} = 0.67 \text{ kg N as urea}\]

\[\text{1 kg N as ammonium sulphate is equivalent to } \frac{66.7}{100} = 0.67 \text{ kg N as urea}\]

B) Comparison of P sources

Comparison of two \(^{32}\text{P}\)-labelled P fertilizers such as superphosphate and nitro-phosphate. (Greenhouse experiment).

Treatments:

\(^{32}\text{P}\)-labelled superphosphate applied at a rate of 50 mg P/kg soil
\(^{32}\text{P}\)-labelled nitro phosphate applied at a rate of 50 mg P/kg soil

Results:

Superphosphate treatment:

\% Pdff = 20%
\% Pdfs = 80%

From the fractional utilization relationship

\[\frac{20}{50} = \frac{80}{X}\]

\[X = \text{available amount of soil P or 200 mg P in superphosphate equivalent units.}\]

Nitro-phosphate treatment:

\% Pdff = 10%
\% Pdfs = 90%

From the fractional utilization relationship

\[\frac{10}{50} = \frac{90}{X}\]

\[X = \text{available amount of soil P or 450 mg P in nitro phosphate units.}\]
Quantitative comparison of superphosphate and nitro-phosphate.

200 mg P as superphosphate = 450 mg P as nitro-phosphate
1 kg P as superphosphate = \( X \)

1 kg P as superphosphate is equivalent to \( \frac{450}{200} = 2.25 \) kg P as nitro-phosphate.

C) Comparison of two formulations of trip's superphosphate

The efficiency of triple superphosphate applied as powder was compared to a granulated formulation of the fertilizer.

Treatments:
- Powdered \( ^{32} \text{P-labelled triple superphosphate} \)
- Granulated \( ^{32} \text{P-labelled triple superphosphate} \)

Rate of application: 40 kg P/ha for both formulations

Results:

Powdered \( ^{32} \text{P-labelled triple super (TSP)} \)
- \( \% \text{Pdf} = 15\% \)
- \( \% \text{PdfS} = 85\% \)

From the fractional utilization relationship

\[
\frac{15}{40} = \frac{85}{X}
\]

\( X = \) available amount of soil P or 227 kg P as powdered TSP units

Granulated \( ^{32} \text{P-labelled TSP} \)
- \( \% \text{Pdf} = 20\% \)
- \( \% \text{PdfS} = 80\% \)

From the fractional utilization relationship

\[
\frac{20}{40} = \frac{80}{X}
\]

\( X = \) available amount of soil P or 160 kg P as granulated TSP units.
Quantitative comparison of powdered and granulated TSP

227 kg P as powdered TSP = 160 kg P as granulated TSP

1 kg P as powdered TSP = X

1 kg P as powdered TSP is equivalent to \( \frac{160}{227} = 0.70 \) kg as granulated TSP.

If the application of 40 kg P/ha as powdered TSP was the farmer's practice and he decides to change the formulation, the farmer should now apply 28 kg P/ha as granulated TSP to get the same amount of fertilizer taken up.

Fertilizer sources which cannot be labelled (Indirect method)

This methodology can be used in the following cases:

a. When it is impossible to label fertilizer sources such as natural products (rock phosphates) and organic materials, ie: guano, green and animal manures, compost, agricultural residues, etc.

b. When it is impractical to label nutrient sources such as the atmospheric \( N_2 \) which can be biologically fixed by field-grown legume crops (see Hardarson and Danso, this volume).

c. When the cost of the required labelled fertilizers for the experiment e.g. study of residual effect, is too high or simply the required labelled fertilizers for a particular study are not available.

The isotope dilution technique is used in this case. It essentially consists of labelling the soil with an isotopically labelled solution and use the plant to measure the isotopic ratio (\( \%^{15}N \) atom excess for nitrogen or specific activity for phosphorus) of the N or P supplied by the labelled soil (soil + solution). This method is usually referred to as "the isotope dilution technique", although this is not an isotope dilution as defined in isotope chemistry.

The isotopic ratio in the fertilized treatment will decrease as a result of the N or P supply from unlabelled source. A standard treatment (without application of the unlabelled source) is also required.
Isotope solutions:

In case of P, a solution of KH$_2$PO$_4$ or NaH$_2$PO$_4$ (at low P concentration: 10-50 ppm P) labelled with $^{32}$P carrier free or a high specific activity orthophosphate solution (available from commercial firms) can be used. Rate of isotope application (irrespective of the rate of application of P) should be about $7.4-18.5 \times 10^6$ Bq (200-500 $\mu$Ci) $^{32}$P/m$^2$ to field plots and about $3.7-7.4 \times 10^6$ Bq (100-200 $\mu$Ci) $^{32}$P/kg soil in greenhouse experiments.

In case of N, any solution of fertilizer N (NH$_4^+$ or NO$_3^-$ labelled with $^{15}$N) can be used. Rate of isotope application (irrespective of the rate of application of N) should be about 0.1 g $^{15}$N/m$^2$ if analysed by mass spectrometry or 0.2 g $^{15}$N/m$^2$ if analysed by optical emission spectrometry.

Experimental treatments: A pair of treatments is required to measure the isotopic ratios of the N or P supplied by the "labelled soil", in presence and absence of the unlabelled source.

Treatment I or standard (In absence of the unlabelled fertilizer source): Labelled soil (soil + isotopically labelled solution).

Treatment II (In presence of the unlabelled source): Labelled soil (soil + isotopically labelled solution) + unlabelled source.

In all cases the "labelling" procedure should be done first by uniformly applying the labelled solution over the soil. Ensure a thorough mixing of the soil with the solution and leave to reach equilibrium. After about 1 week the unlabelled fertilizer source should be applied.
Example:

Agronomic evaluation of a rock phosphate i.e. quantitative comparison of a rock phosphate with superphosphate (Fried, 1954; Zapata et al., 1986).

Treatments:

I : Soil + $^{32}$P-labelled solution
II : Soil + superphosphate (60 kg P/ha) + $^{32}$P-labelled solution
III : Soil + rock phosphate (200 kg P/ha) + $^{32}$P-labelled solution

Results:

Specific activities (S.A.) of the harvested plant material per treatment.

I : S.A. plant = 2000 dpm/mg P
II : S.A. plant = 1200 dpm/mg P
III : S.A. plant = 1400 dpm/mg P

Calculations:

\[
\% \text{ Pdff} = \frac{\text{S.A. plant sample}}{\text{S.A. labelled fertilizer}} \times 100 \tag{26}
\]

\[
\% \text{ Pdfl} = \frac{\text{S.A. plant sample}}{\text{S.A. labelled source}} \times 100 \tag{27}
\]

Since S.A. labelled source = S.A. labelled soil

\[
\% \text{ Pdfl} = \frac{\text{S.A. plant sample}}{\text{S.A. labelled soil}} \times 100 \tag{28}
\]

and S.A. labelled soil = S.A. of the plant in treatment I.

Treatment II

\[
\% \text{ Pdfl} = \frac{1200}{2000} \times 100
\]

\[= 60\%\]

\[
\% \text{ Pdf. unl. fert. (super)} = 100 - 60
\]

\[= 40\%\]
From the fractional utilization relationship
\[
\frac{40}{60} = \frac{60}{X}
\]

\(X\) = available amount of soil P or 90 kg P/ha as superphosphate equivalent units.

**Treatment III**

\[
\%\ P\text{dfl} = \frac{1400}{2000} \times 100
\]

\(= 70\%\)

\(\%\ P\text{dfl unl. fert (rock phosphate)} = 100 - 70\)

\(= 30\%\)

From the fractional utilization relationship
\[
\frac{30}{200} = \frac{70}{X}
\]

\(X\) = available amount of soil P or 467 kg P/ha as rock phosphate equivalent units.

**Quantitative comparison of rock phosphate and superphosphate**

90 kg P as superphosphate = 467 kg P as rock phosphate

1 kg P as superphosphate = \(X\)

1 kg P as superphosphate is equivalent to \(\frac{467}{90} = 5.2\) kg P as rock phosphate.

**Agronomic evaluation of guano materials**

**Objective:** To assess the plant-available amounts of N in guano materials from different origin.

**Treatments:**

Pots containing 2 kg air dried alkaline Seibersdorf soil were used for the following treatments:

I \(^{15}\)N-labelled ammonium sulphate solution (\(^{15}\)N-A.S. at 100 mg N/pot)

II \(^{15}\)N-A.S. + ammonium sulphate (A.S. at 200 mg N/pot)

III \(^{15}\)N-A.S. + guano (A) from Peru (GP at 390 mg N/pot)

IV \(^{15}\)N-A.S. + guano (B) from Zaire (GZ at 390 mg N/pot)
Experimental results:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dry matter yield g/pot</th>
<th>N yield mg N/pot</th>
<th>Ndff %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.28</td>
<td>135</td>
<td>29.4</td>
</tr>
<tr>
<td>2</td>
<td>7.62</td>
<td>187</td>
<td>17.8</td>
</tr>
<tr>
<td>3</td>
<td>8.15</td>
<td>247</td>
<td>11.3</td>
</tr>
<tr>
<td>4</td>
<td>7.99</td>
<td>224</td>
<td>13.5</td>
</tr>
</tbody>
</table>

Calculations:

\[ \% \text{Ndff} = \frac{\% 15\text{N a.e. plant sample}}{\% 15\text{N a.e. labelled soil}} \times 100 \]

\[ \% 15\text{N a.e. labelled soil} = \% 15\text{N a.e. plant in treatment I.} \]

\[ \% \text{Ndff} = \frac{\% 15\text{N a.e. plant sample-treatment unlabelled source}}{\% 15\text{N a.e. plant sample - treatment I}} \times 100 \]

where unlabelled source = guano, and

\[ \% \text{Ndfg} = 100 - \% \text{Ndff} \]

or

\[ \% \text{Ndfg} = \{ 1 - \frac{\% 15\text{N a.e. plant sample-treatment unlabelled source}}{\% 15\text{N a.e. plant sample-treatment I}} \} \times 100 \]

or the same:

\[ \% \text{Ndfg} = \{ 1 - \frac{\% \text{Ndff - treatment unlabelled source}}{\% \text{Ndff - treatment I}} \} \]

**Treatment II**

For the commercial ammonium sulphate (AS):

\[ \% \text{Nd AS} = \{ 1 - \frac{17.8}{29.4} \} \times 100 \]

\[ = 39.5\% \]
From the fractional utilization relationship:

\[
\frac{39.5}{200} = \frac{60.5}{X}
\]

\(X\) = available amount of soil N or 306 mg N/pot as ammonium sulphate equivalent units.

**Treatment III**

For the guano of Peru (GP):

\[
\% \text{ Ndf GP} = \left\{ 1 - \frac{11.3}{29.4} \right\} \times 100
\]

From the fractional utilization relationship

\[
\frac{61.6}{420} = \frac{38.4}{X}
\]

\(X\) = available amount of soil N or 262 mg N/pot as guano of Peru equivalent units.

**Treatment IV**

For the guano of Zaire (GZ):

\[
\% \text{ Ndf GZ} = \left\{ 1 - \frac{13.5}{29.4} \right\} \times 100
\]

\(= 54.1\%\)

From the fractional utilization relationship

\[
\frac{54.1}{390} = \frac{54.9}{X}
\]

\(X\) = available amount of soil N or 330 mg N/pot as guano of Zaire equivalent units.

Quantitative comparison of guano materials and ammonium sulphate.

**a) In terms of nutrient N:**

306 mg N as ammonium sulphate = 262 mg N as guano of Peru.

306 kg N = 262 kg N

1 kg N as ammonium sulphate = \(X\)
1 kg N as ammonium sulphate is equivalent to \( \frac{262}{306} \) or 0.86 kg N as guano of Peru.

306 mg N as ammonium sulphate = 330 mg N as guano of Zaire

306 kg N as ammonium sulphate = 330 kg N as guano of Zaire

1 kg N as ammonium sulphate = X

1 kg N as ammonium sulphate is equivalent to \( \frac{330}{306} \) or 1.08 kg N as guano of Zaire.

b) In terms of product

\[
1 \text{ kg N as A.S.} = 0.86 \text{ kg N as guano of Peru}
\]

\[
1 \times \frac{100}{21} \text{ kg A.S.} = 0.86 \times \frac{100}{4.72} \text{ kg GP}
\]

4.76 kg A.S. = 18.22 kg guano of Peru

1 kg ammonium sulphate = 3.8 kg guano of Peru

1 kg N as A.S. = 1.08 kg N as guano of Zaire

\[
1 \times \frac{100}{21} \text{ kg A.S.} = 1.08 \times \frac{100}{13.55} \text{ kg GZ}
\]

4.76 kg A.S. = 7.97 kg guano of Zaire

1 kg ammonium sulphate = 1.67 kg guano of Zaire

The "A value technique" may also be used to estimate the available amounts of nutrient in fertilizer sources which cannot be labelled. In this case the N(P) in the plant derived from a labelled fertilizer must be determined in presence and absence of the unlabelled fertilizer source. Similarly to the "isotope dilution technique", the following treatments are required:

Treatment I or standard (In absence of the unlabelled fertilizer source): Soil + isotopically-labelled fertilizer. This treatment is used to estimate the A value of the soil.

A value treatment I = A soil

Treatment II (In presence of the unlabelled fertilizer source): Soil + unlabelled fertilizer source + isotopically-labelled fertilizer. From this treatment the combined A value of the soil and the unlabelled fertilizer source can be estimated.
A value treatment II = A soil + A unl. fert. source

Finally the A value of the unlabelled fertilizer source is obtained by difference:

\[ A \text{ unl. fert. source} = (A \text{ soil} + A \text{ unl. fert. source}) - (A \text{ soil}) \]

Example:

Agronomic evaluation of a rock phosphate i.e. quantitative comparison of a rock phosphate with ordinary superphosphate (OSP).

Treatments:

I : Soil+\(^{32}\)P-OSP (50mg P/kg soil)
II: Soil+rock phosphate (500mg P/kg soil)+\(^{32}\)P-OSP (50mg P/kg soil)

Results:

% Pdff in the harvested plant material is determined in each treatment.

**Treatment I**

% Pdff = 30, and % Pdfs = 100-30
\[ % \text{ Pdfs} = 70\% \]

From the fractional utilization relationship:

\[ \frac{30}{50} = \frac{70}{A \text{ soil}} \]

A soil = 117 mg P/kg soil as super equivalent units.

**Treatment II**

% Pdff = 20%, and 100-% Pdff = 80%

From the fractional utilization relationship:

\[ \frac{20}{50} = \frac{80}{A \text{ soil} + A \text{ rock}} \]

A soil + A rock = 200 mg P/kg soil as superphosphate equivalent units.
From treatments I and II:

\[ A \text{ rock} = (A \text{ soil} + A \text{ rock}) - (A \text{ soil}) \]

\[ A \text{ rock} = 200 - 117 \]

\[ = 83 \text{ mg P/kg soil as superphosphate equivalent units} \]

Quantitative comparison of rock phosphate and superphosphate:

\[ 500 \text{ mg P as rock phosphate} = 83 \text{ mg P as superphosphate} \]

\[ 500 \text{ kg P} \]

\[ \times \]

\[ = 83 \text{ kg P as superphosphate} \]

\[ = 1 \text{ kg P as rock phosphate} \]

1 kg P as superphosphate is equivalent to \( \frac{500}{83} = 6 \) kg P as rock phosphate.

Availability of nitrogen in Azolla

Objective: To assess the plant-available amounts of N in Azolla applied to flooded rice growing in the field (Kumarasinghe et al., 1986).

Treatments:

I - Soil + \(^{15}\)N-urea* (100 kg N/ha)

II - Soil + \(^{15}\)N-urea* (100 kg N/ha) + unlabelled Azolla (250 kg N/ha)

Experimental results:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant parts of rice</th>
<th>Dry Matter Yield (ton/ha)</th>
<th>Nitrogen Yield (kg N/ha)</th>
<th>Ndff %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Straw</td>
<td>7.5</td>
<td>45</td>
<td>25.4</td>
</tr>
<tr>
<td></td>
<td>Spikes</td>
<td>4.5</td>
<td>62</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>12.0</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Straw</td>
<td>13.3</td>
<td>136</td>
<td>15.1</td>
</tr>
<tr>
<td></td>
<td>Spikes</td>
<td>8.2</td>
<td>122</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>21.5</td>
<td>258</td>
<td></td>
</tr>
</tbody>
</table>
Treatment I

\[ \% \text{Ndff} = \frac{(45 \times 25.4/100) + (62 \times 24.1/100)}{107} \times 100 \]

\[ = \frac{11.43 + 14.94}{107} \times 100 \]

\[ = 24.6\% \text{ (weighted average for the whole plant)} \]

and \( \% \text{Ndfs} = 100 - 24.6 \)
\[ \% \text{Ndfs} = 75.4\% \]

From the fractional utilization relationship:

\[ \frac{24.6}{100} = \frac{75.4}{A \text{ soil}} \]

\[ A \text{ soil} = 307 \text{ kg N/ha as urea equivalent units.} \]

Treatment II

\[ \% \text{Ndff} = \frac{(136 \times 15.1/100) + (122 \times 13.6/100)}{258} \times 100 \]

\[ = \frac{20.54 + 16.59}{258} \times 100 \]

\[ = 14.4\% \text{ (weighted average for the whole plant)} \]

and \( \% \text{Ndfs} = 100 - \% \text{Ndff} = 85.6\% \]

From the fractional utilization relationship

\[ \frac{14.4}{100} = \frac{85.6}{A \text{ soil} + A \text{ Azolla}} \]

\[ A \text{ soil} + A \text{ Azolla} = 594 \text{ kg N/ha as urea equivalent units.} \]

From the treatments I and II it may be inferred that:

\[ A \text{ Azolla} = (A \text{ soil} + A \text{ Azolla}) - (A \text{ soil}) \]
\[ A \text{ Azolla} = 594 - 307 \]
\[ = 287 \text{ kg N/ha as urea equivalent units.} \]
The fractional utilization relationship is as follows:

\[ \frac{14.4}{100} = \frac{\%Ndfs}{307} = \frac{\%NdF\text{ Azolla}}{287} \]

\[ \%Ndfs = 307 \times \frac{14.4}{100} = 44.2\% \]

\[ \%NdF\text{ Azolla} = 287 \times \frac{14.4}{100} = 41.3\% \]

and \( \%Ndff = 14.4\% \) (experimentally determined).

Since the total N yield = 258 kg N/ha

\[ \text{Azolla N yield} = 258 \times \frac{41.3}{100} = 107 \text{ kg N/ha} \]

\[ \text{Soil N yield} = 258 \times \frac{44.2}{100} = 114 \text{ kg N/ha} \]

\[ \text{Fertilizer N yield} = 258 \times \frac{14.4}{100} = 37 \text{ kg N/ha} \]

The % recovery of Azolla-N is calculated as follows:

\[ \% \text{ recovery} = \frac{\text{Azolla N yield}}{\text{Azolla N applied rate}} \times 100 \]

\[ = \frac{107}{250} \times 100 \]

\[ = 42.8\% \]

Quantitative comparison of Azolla and urea:

250 kg N as Azolla = 287 kg N as Urea

\[ X = 1 \text{ kg N as Urea} \]

\[ X = 0.87 \text{ kg N as Azolla} \]

1 kg N as Urea = 0.87 kg N as Azolla

\[ 1 \times \frac{100}{46} \text{ kg urea} = 0.87 \times \frac{100}{4.45} \text{ kg as Azolla} \]

2.17 kg Urea = 19.6 kg Azolla

1 kg Urea = 9 kg Azolla

The use of the \( ^{15}\text{N} \) methodology for the quantitative measurement of the biological nitrogen fixation.
The principle of the $^{15}$N methodology for quantitatively measuring the symbiotic nitrogen fixation by field-grown legumes is similar to that before-mentioned for rock phosphate or any other unlabelled fertilizer source. This aspect is illustrated by Hardarson and Danso (this volume).

REFERENCES


USE OF $^{15}$N METHODOLOGY TO ASSESS BIOLOGICAL NITROGEN FIXATION

G. HARDARSON
Soil Science Unit,
FAO/IAEA Programme,
Agency's Laboratory,
International Atomic Energy Agency,
Seibersdorf

S.K.A. DANSO
Joint FAO/IAEA Division of Nuclear Techniques
in Food and Agriculture,
International Atomic Energy Agency,
Vienna

INTRODUCTION

Legumes play an important part in the diets of most of the people of the world, and they are second only to cereals as a source of human and animal food. Grain legumes are a particularly important source of protein. Although in terms of dry matter production, legumes (food legumes and leguminous oil seeds) account for only 10% of the combined world yield of cereals and legumes, they constitute as much as 24% of the total protein yield of these crops because of the latter's high protein content (Table 1).

Table 1: Protein content of cereals and grain legumes (Sinha, 1977)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Average protein content %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cereals</strong></td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>12.3</td>
</tr>
<tr>
<td>Rice</td>
<td>8.3</td>
</tr>
<tr>
<td>Maize</td>
<td>8.9</td>
</tr>
<tr>
<td>Barley</td>
<td>8.6</td>
</tr>
<tr>
<td>Sorghum</td>
<td>11.0</td>
</tr>
<tr>
<td>Oats</td>
<td>11.0</td>
</tr>
<tr>
<td><strong>Grain legumes</strong></td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td>38.0</td>
</tr>
<tr>
<td>Groundnut</td>
<td>25.6</td>
</tr>
<tr>
<td>Dried beans</td>
<td>22.3</td>
</tr>
<tr>
<td>Peas</td>
<td>24.1</td>
</tr>
<tr>
<td>Broad beans</td>
<td>25.1</td>
</tr>
<tr>
<td>Chickpeas</td>
<td>24.0</td>
</tr>
<tr>
<td>Lentils</td>
<td>28.0</td>
</tr>
</tbody>
</table>
One of the most important characteristics of legumes are their ability in symbiosis with *Rhizobium* bacteria to fix atmospheric nitrogen for growth. Legumes having effective biological nitrogen fixation can therefore be grown without nitrogen fertilizer. Having these characteristics, they are particularly important in the developing countries due to the high cost and/or restricted availability of nitrogen fertilizer.

For proper management and a full realization of the benefits of this plant-microbial association, it is necessary to estimate how much nitrogen is fixed under different conditions in the field. It is only after this is known that various factors can be manipulated so as to increase the amount and proportion of N a plant derives from biological fixation. A suitable method for accurately measuring the amount of N crops derive from fixation is therefore an important requirement in any programme aimed at maximizing biological nitrogen fixation.

There are several methods available to measure N\textsubscript{2} fixation (Bergersen, 1980) based on (1) increment in N yield and plant growth, (2) nitrogen balance (3) acetylene reduction and (4) the use of isotopes of N. Only isotopic methods will be illustrated here.

**USE OF ISOTOPES OF N TO MEASURE NITROGEN FIXATION**

Nitrogen has six isotopes, varying in atomic mass from 12 to 17 (Table 2). Of these, \(^{14}\text{N}\) and \(^{15}\text{N}\) are stable isotopes, while the rest are radioactive. (See Axmann and Zapata, this volume). \(^{15}\text{N}_2\) as well as \(^{15}\text{N}\) enriched or depleted inorganic or organic fertilizers have been widely used for quantifying nitrogen fixed.

<table>
<thead>
<tr>
<th>Mass number</th>
<th>Natural abundance</th>
<th>Half life</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>-</td>
<td>0.0125 s</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>10.05 min</td>
</tr>
<tr>
<td>14</td>
<td>99.634</td>
<td>(stable)</td>
</tr>
<tr>
<td>15</td>
<td>0.366</td>
<td>(stable)</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>7.36 s</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>4.14 s</td>
</tr>
</tbody>
</table>

Table 2. Isotopes of nitrogen
The use of $^{15}\text{N}$ as a tracer is based on the occurrence in nature of $^{14}\text{N}$ and $^{15}\text{N}$ in an almost constant ratio of $272 \pm 0.3$ to 1 yielding $0.3663 \pm 0.0004$ atom % $^{15}\text{N}$. The amount of $^{15}\text{N}$ in a sample is conveniently expressed as % $^{15}\text{N}$ atom excess (% $^{15}\text{N}$ a.e.) over the natural abundance in atmospheric nitrogen of 0.3663. In N isotopic tracer studies, the system under investigation is supplied with materials containing $^{15}\text{N}/^{14}\text{N}$ ratios measurably different from the $^{15}\text{N}$ natural abundance. It is also essential that the nitrogen isotope ratio should again be measurably different from $^{15}\text{N}$ natural abundance at the time the system under investigation is sampled. In the case of plants, for example, the uptake of $^{15}\text{N}$ enriched fertilizer added to soil will result in a $^{15}\text{N}/^{14}\text{N}$ ratio greater than 0.3663% within the plant. The extent of increase in the $^{15}\text{N}/^{14}\text{N}$ ratio in the plant over that of natural abundance is a reflection of the extent of uptake of the labelled $^{15}\text{N}$ fertilizer, while a decrease in the % $^{15}\text{N}$ atom excess of the fertilizer nitrogen within the plant is an indication of the extent to which the plant took up N from available sources of unlabelled N. Isotopes of N may be used in the gaseous $\text{N}_2$ form, or as labelled substrates in solid or liquid forms.

**USE OF $^{15}\text{N}_2$**

The earliest application of $^{15}\text{N}_2$ in $\text{N}_2$ fixation studies was by Burris and Miller (1941). This method has been used to provide direct evidence for $\text{N}_2$ fixation since the $^{15}\text{N}$ concentration in plants exposed to $^{15}\text{N}_2$ is greater than the 0.3663% natural abundance if fixation occur. The extent to which $^{15}\text{N}$ is detected in the plant provides an estimate of the proportion of the plant's N that was derived from fixation, and is thus a direct method for quantifying $\text{N}_2$ fixed. The use of $^{15}\text{N}_2$ involves the enclosure of plants in chambers filled with the enriched gas (Witty and Day, 1978). The environment within the chamber is, however, different from that in a field situation. Also, the plants cannot be confined in these chambers for long periods. Results obtained from such studies therefore tend to be instantaneous and subject to errors associated with extrapolating data from short-term studies to a growing season which involves diurnal, daily and seasonal variations (Knowles, 1980).
USE OF $^{15}$N ENRICHED FERTILIZERS OR SUBSTRATES (ISOTOPE DILUTION METHOD)

The method involves the growth of $N_2$ fixing (F) and non-fixing reference (NF) plants in soil fertilized with $^{15}$N enriched inorganic or organic fertilizers. It is based on differential dilution in the plant of $^{15}$N-labelled fertilizer by soil and fixed nitrogen (McAuliffe et al., 1958; Fried and Broeshart, 1975; Fried and Middelboe, 1977). It provides an integrated measurement of amount of fixed $N$ accumulated by a crop over the growing season.

To be able to measure $N_2$ fixation of a legume crop, a plot of legume and another with a non-fixing reference crop are needed. One reference plot in each replication is sufficient if varieties or strains of Rhizobium are being tested. However, if $P$ application or time of harvesting are being studied, the reference crop has to be treated the same way as the fixing crop. In this case, one plot of reference crop is needed for each plot of fixing crop. 1-3 m$^2$ isotope plots with at least 20-30 plants to be harvested per plot, are usually sufficient. Approximately 0.1 g $^{15}$N/m$^2$, i.e., 20 kg N/ha of 5% $^{15}$N a.e. or 100 kg N/ha of 1% $^{15}$N a.e. is usually enough for detection. Urea, ammonium nitrate or ammonium sulphate fertilizer can be applied in a solid or liquid form (fertilizer dissolved in at least 500 ml water/m$^2$). Precaution should be taken not to apply the fertilizer when the soil temperature is very high due to direct ammonia volatilization.

At the time of harvesting, the plots of reference crop should always be harvested at the same time as the plots with fixing crops. The pods and straw have usually very different % $N$ and % $^{15}$N a.e. Therefore it is not possible to take directly representative subsample from those plant parts when mixed. The plants should therefore be separated into pods and straw. These parts are then weighed and subsampled after chopping into small fragments. Material with lower % $^{15}$N a.e. should be chopped first to minimize contamination from one sample to another. In any case, the forage chopper, if used, has to be cleaned thoroughly between treatments.

Subsamples should be ground after drying at 70°C. These samples can then be analysed for total $N$ by the Kjeldahl procedure. The N-15/N-14 ratio can be analysed by either emission or mass spectrometer as described by Axmann and Zapata (this volume).
After the analyses of $^{15}\text{N}$ abundance in the plant and fertilizer samples, $^{15}\text{N}$ atom excess has to be calculated by subtracting the $^{15}\text{N}$ natural abundance (0.3663%) from the $^{15}\text{N}$ abundance in the sample. The $^{15}\text{N}$ atom excess values are used for all the following calculations.

$^{15}\text{N}$ derived from fertilizer (% Ndff) is the first derived value as shown by Example 1.

Example 1

In a field experiment 50 kg N/ha of 2.501 $^{15}\text{N}$ atom excess ammonium sulphate was applied to a cereal crop. At the end of the growing season plant sample from the harvested material had 0.534 $^{15}\text{N}$ atom excess. What was the $^{15}\text{N}$ derived from fertilizer (% Ndff)?

Calculation:

$$\text{% Ndff} = \frac{^{15}\text{N atom excess (plant)}}{^{15}\text{N atom excess (fertilizer)}} \times 100 \quad (1)$$

$$\text{% Ndff} = \frac{0.534}{2.501} \times 100 = 21\%$$

21% of the N in the plant was derived from fertilizer and the remaining 79% was derived from soil N.

Some type of calculation can be made for legume crops as illustrated in the next example.

Example 2

20 kg N/ha of 5.231 $^{15}\text{N}$ atom excess was applied to a fixing (F) and a non-fixing (NF) crops in a field experiment. Plant samples from the harvested materials yielded 0.702 and 1.251 $^{15}\text{N}$ atom excess for F and NF crops, respectively. What were the % Ndff for the two crops?
Calculation:

\[ \% \text{Ndff} = \left( \frac{\% \text{15 N a.e. (plant)}}{\% \text{15 N a.e. (fert.)}} \right) \times 100 \]

\[ \% \text{Ndff}_F = \frac{0.702}{5.231} \times 100 = 13.4\% \]

\[ \% \text{Ndff}_N = \frac{1.251}{5.231} \times 100 = 23.9\% \]

In the NF crop the remaining 76% was derived from soil N. However in the F crop the remaining 87% were derived from soil (\%Ndfs) and atmosphere (\%Ndfa) through biological nitrogen fixation as

\[ \% \text{Ndff}_F + \% \text{Ndfs}_F + \% \text{Ndfa} = 100 \]

The question therefore remains what were the proportion derived from soil and air in the F crop.

To be able to calculate the relative proportion derived from air and soil the following assumption has to be introduced:

**Assumption:**

*It is assumed that both non-fixing and fixing crops take up N from soil and fertilizer in the same ratio i.e.*

\[ \frac{\% \text{Ndff}}{\% \text{Ndff}} = \frac{\% \text{Ndff}}{\% \text{Ndff}} \]

\[ \frac{\% \text{Ndfs}}{\% \text{Ndfs}} \]

(2)

Using this equation the calculation of example 2 can be continued as illustrated in the following table:

<table>
<thead>
<tr>
<th></th>
<th>% 15N a.e.</th>
<th>% Ndff</th>
<th>% Ndfs</th>
<th>% Ndff</th>
<th>% Ndfa</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF</td>
<td>1.251</td>
<td>23.9</td>
<td>76.1</td>
<td>0.314*</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0.702</td>
<td>13.4</td>
<td>42.7**</td>
<td>0.314*</td>
<td>43.9***</td>
</tr>
<tr>
<td>Fert.</td>
<td>5.231</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
According to the above assumption:

\[
\frac{% Ndff_{NF}}{% Ndfs_{NF}} = \frac{23.9}{76.1} = 0.314 = \frac{% Ndff_{F}}{% Ndfs_{F}}
\]

Thus

\[
\frac{% Ndfs_{F}}{0.314} = 13.4 = 42.7
\]

and

\[
% Ndfa = 100 - % Ndff_{F} - % Ndfs_{F}
\]

\[
% Ndfa = 100 - 13.4 - 42.7
\]

\[
% Ndfa = 43.9
\]

In this way the proportions of N from all available sources has been quantified i.e. for NF

\[
% Ndff = 23.9
\]

\[
% Ndfs = 76.1
\]

and for F

\[
% Ndff = 13.4
\]

\[
% Ndfs = 42.7
\]

\[
% Ndfa = 43.9
\]

The accuracy and precision of the isotope dilution method depends to a great extent on selecting a suitable NF reference crop. The selection of the appropriate reference plant is therefore crucial, and it is essential to observe the following:

(i) That the reference crop does not itself fix nitrogen. This can, if necessary, be checked very quickly, using the acetylene reduction assay.

(ii) The rooting depths of both reference and fixing crops should be similar, or both crops should derive all of their N from the same zone.

(iii) Both N\textsubscript{2} fixing and standard crops should go through similar growth or physiological stages, and mature about the same time.
Both $N_2$ fixing and standard crops should be planted and harvested at the same time.

Both crops should be affected in similar fashion by changes in environmental conditions, such as temperature and water, during growth period.

For estimating $N_2$ fixed in grain legumes, the following NF reference crops have been successfully used:

1) A non-legume, non-fixing plant.
2) A non-nodulating legume plant.
3) An uninoculated legume plant in soils devoid of the appropriate strains of Rhizobium.

There are three main variations in the technique: (1) the isotope dilution method (ID) (McAuliffe et al., 1958; Fried and Middelboe, 1977), (2) the A-value method (AV) (Fried and Broeshart, 1975) and (3) the single treatment method (ST) (Fried and Broeshart, 1981).

Method (1) The isotope dilution method

This is the case when both fixing and reference plants are grown on soil to which the same amount of fertilizer having the same $^{15}$N enrichment have been applied as illustrated in previous Example 2. Thus, in the absence of any supply of N other than soil and $^{15}$N labelled fertilizer, a fixing plant and a non-fixing reference plant will contain the same ratio of $^{15}$N/$^{14}$N, since they are taking N of similar $^{15}$N/$^{14}$N composition, but not necessarily the same total quantity of N. In both plants, the $^{15}$N/$^{14}$N ratio within the plant is lowered by the N absorbed from the unlabelled soil. However, in the presence of $N_2$, the fixing plant further lowers the ratio of $^{15}$N/$^{14}$N due to incorporation of N from unlabelled air, while this does not occur in the non-fixing plant. The extent to which the $^{15}$N/$^{14}$N ratio in the fixing crop is decreased, relative to the non-fixing plant, is therefore an indication of $N_2$ fixing ability, and can be used to estimate $N_2$ fixed in the field.

The determination of $N_2$ fixation using this approach is depicted in Fig. 1 by a fictitious example. By using $^{15}$N labelled fertilizer, 50% of the N in the NF reference crop was derived from the applied fertilizer. Since there are only two sources of N available to this crop,
It follows from equation (3) that the other half or 50% of the N in plant came from soil. This then establishes that the ratio of soil to fertilizer N available to the non-fixing plant was 1:1 in this example.

For the N₂ fixing crop (F) in Fig. 1, there is a third source of N available to the plants, i.e., N₂ from the atmosphere. The total N in plant can therefore be represented by the following equation:

\[ \% \text{Ndff}_F + \% \text{Ndfs}_F + \% \text{Ndfa} = 100 \]  
(4)

or

\[ c + d + e = 100 \]
The non-fixing reference crop took up N from soil and fertilizer in the ratio 1, and it is assumed as shown in equation (2) that the same occurs in the fixing crop.

That is:

\[ \frac{a}{b} = \frac{c}{d} \]

In the example % Ndff in the fixing crop was 25%. Therefore, according to equation (2) the % Ndfs in the fixing crop is also 25%. The rest of the N taken up (50%) was derived from atmosphere, since according to equation (4):

\[ % \text{Ndfa} = 100 - (% \text{Ndff} + % \text{Ndfs}) \]

The equation to calculate percentage N derived from atmosphere are derived from equations 2, 3 and 4 as follows:

From (4)

\[ e = 100 - c - d \]  \hspace{1cm} (5)

and from (2)

\[ d = \frac{c}{a} \times b \]  \hspace{1cm} (6)

where according to (3)

\[ b = 100 - a \]

From (3) and (6)

\[ d = \frac{c}{a} \times (100 - a) \]

or

\[ d = \frac{100 \cdot c}{a} - c \]  \hspace{1cm} (7)

From (5) and (7)

\[ e = 100 - c - \left( \frac{100 \cdot c}{a} - c \right) \]
or

\[ e = (1 - \frac{c}{a}) \times 100 \quad (8) \]

or

\[ % \text{Ndfa} = (1 - \frac{\% \text{Ndff}_F}{\% \text{Ndff}_N}) \times 100 \]

Since according to equation 1

\[ % \text{Ndff} = \frac{\% \text{N}_{\text{atom excess of sample}}}{\% \text{N}_{\text{atom excess of fertilizer}}} \times 100 \]

Then from (1) and (8)

\[ % \text{Ndfa} = (1 - \frac{\% \text{N}_{\text{a.e.}}_F}{\% \text{N}_{\text{a.e.}}_N}) \times 100 \quad (9) \]

Amount of \( \text{N}_2 \) fixed can be calculated according to:

\[ \text{Amount of } \text{N}_2 \text{ fixed} = \frac{\% \text{Ndfa} \times \text{total N in fixing crop}}{100} \quad (10) \]

The use of these formulae is shown in Tables 3 and 4.
Table 3. The following data were recorded for nodulating (F) and non-nodulating (NF) soybean both of variety Chippewa at the Seibersdorf Laboratory, Austria, in 1981. 20 kg N/ha of $^{15}$N labelled fertilizer was applied to the fixing and non-fixing crops. Only one replicate from five is shown in this example.

<table>
<thead>
<tr>
<th>Fixing crop</th>
<th>Dry matter yield $^{1)}$ (kg/ha)</th>
<th>N $^{2)}$ (%)</th>
<th>N yield $^{4)}$ (kg/ha)</th>
<th>$^{15}$N atom excess $^{3)}$ (%)</th>
<th>Ndff $^{5)}$ (%)</th>
<th>N fert. yield $^{6)}$ (kg/ha)</th>
<th>Ndfa $^{8)}$ (%)</th>
<th>Fixed N $^{9)}$ (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stems</td>
<td>4478</td>
<td>0.63</td>
<td>38.2</td>
<td>0.152</td>
<td>3.16</td>
<td>0.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>2743</td>
<td>1.90</td>
<td>52.1</td>
<td>0.158</td>
<td>3.28</td>
<td>1.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pods</td>
<td>1867</td>
<td>2.58</td>
<td>48.2</td>
<td>0.132</td>
<td>2.74</td>
<td>1.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.05 $^{7)}$</td>
<td>3.92</td>
<td>26</td>
</tr>
</tbody>
</table>

Other values needed for the calculation:  
a) % $^{15}$N atom excess of fertilizer: 4.81  
b) % Ndff $^{\text{NF}}$: 4.14 (calculated by the same method as % Ndff for fixing crop).  

see Table 4
Table 4: Measurements which were made as well as the formulae used to calculate % Ndfa and amount of fixed N in Table 3

Measured values:

1) Dry matter yield (D.M.) of pods and straw (kg/ha)
2) % N of each plant part in 1)
3) % $^{15}$N atom excess of each plant part in 1) and of fertilizer applied

Calculated values:

4) N yield (kg/ha) of each plant part = \( \frac{D.M. \text{ of each pl. part} \times \% N}{100} \)
5) \( \% \text{Ndff} = \frac{\% 15 \text{N atom excess of sample}}{\% 15 \text{N atom excess of fertilizer}} \times 100 \)
6) N-fert. yield (kg/ha) = \( \frac{N \text{ yield (kg/ha)} \times \% \text{Ndff}}{100} \)
7) \( \% \text{Ndff} \) (weighed average) = \( \frac{\text{Total N fert. yield}}{\text{total N yield}} \) x 100
8) \( \% \text{Ndfa} = \left(1 - \frac{\% \text{Ndff}_{F}}{\% \text{Ndff}_{NF}}\right) \times 100 \)
or
\( \% \text{Ndfa} = \left(1 - \frac{\% 15 \text{N atom excess}_{F}}{\% 15 \text{N atom excess}_{NF}}\right) \times 100 \)
9) $N_2$ fixed (kg/ha) = \( \frac{\% \text{Ndfa} \times \text{total N in fixing crop}}{100} \)
Example 2 can also be calculated using the above equations

\[
\% \text{ Ndff} = (1 - \frac{\% \text{ Ndff}_F}{\% \text{ Ndff}_N}) \times 100
\]

\[
\% \text{ Ndff} = (1 - \frac{13.4}{23.9}) \times 100 = 43.9\%
\]

or

\[
\% \text{ Ndff} = (1 - \frac{15 \text{ N a.e.}_F}{15 \text{ N a.e.}_N}) \times 100
\]

\[
\% \text{ Ndff} = (1 - \frac{0.702}{1.251}) \times 100 = 43.9\%
\]

The assumption made in equation 2 is the only assumption made in Method 1. It is assumed that both fixing and non-fixing crops take up nitrogen from soil and fertilizer in the same ratio. For this to be true the fixing and the non-fixing reference crops have to match and the following conditions have to be met (Witty, 1984):

a) Either fertilizer distribution is even with depth or that the legume and reference crops have spatially similar nutrient uptake profiles.

b) It is assumed that the contribution of seed N in negligible, which is not always true especially if the plants are harvested early in the growing season.

c) It is implicit in the calculation that the enrichment of plant available soil N remains constant with time or that the legume and control have similar N uptake patterns. In practice when fertilizer N is added as a single application the enrichment of plant available soil N declines with time; and this decline can vary between the legume and the control plant. Depending on whether the control takes up soil nitrogen faster or slower than the legume, the calculated nitrogen fixation rate will be greater or less than the true value (Witty, 1983). Errors due to making this assumption may be reduced by use of slow-release N fertilizer and by choice of a control plant which closely parallels the legume in its nitrogen uptake. (Witty, 1984).
The sensitivity of the $^{15}$N methodology to measure symbiotic nitrogen fixation

Methods based on the dilution in the plant of $^{15}$N labelled fertilizer by N derived from atmosphere and soil seem to offer a potentially accurate methods to quantify symbiotic nitrogen fixation. Variations are however often found depending on the non-fixing standard crop (Wagner and Zapata, 1982). This has been found to be mainly due to differences in N uptake patterns of the legume and control combinations, together with a decrease in the $^{15}$N/$^{14}$N ratio of the substrate with time (Witty, 1983).

It has been observed at the Seibersdorf Laboratory that the $^{15}$N methodology seems to be particularly accurate when large proportions of the N in the fixing plant is derived from atmosphere (Reichardt et al., 1987, Hardarson et al., 1988). This prompt us to model the percentage of N derived from atmosphere in relation to $^{15}$N enrichment in the fixing and non-fixing standard crops and to investigate where major errors in estimates of N$_2$ fixation can be expected.

As shown previously, percentage N derived from atmosphere (e) can be calculated according to:

$$e = (1 - \frac{c}{a}) \times 100$$

where c and a are either % $^{15}$N atom excess or % Ndff of the fixing and non-fixing crops, respectively.

Using this equation modelled curves for various c and a of both fixing and non-fixing crops are shown in Fig. 2. When c is 1% e increases very rapidly up to 80% with increased value of a. At higher c values differences in a will not affect e to the same extent.

A 10% coefficient of variation of a (% Ndff or % $^{15}$N a.e. of reference crop) produces much larger variation in e (% Ndff) when it is small (Fig. 2 and Table 5).
FIG. 2. Modelled curves for $e$ (% Ndfa) at various $c$ (% Ndff,%) and $a$ (% Ndff,f) values.

Table 5: Calculated values for $e$ (% Ndfa) from various values of $c$ (% 15N a.e. or % Ndff of fixing crop) when $a$ (% 15N a.e. or % Ndff of reference crop) has 10% coefficient or variation.

<table>
<thead>
<tr>
<th>Example</th>
<th>$a$</th>
<th>$c$</th>
<th>$e$</th>
<th>range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 ± 2</td>
<td>18</td>
<td>0 - 18</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>20 ± 2</td>
<td>12</td>
<td>33 - 45</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>20 ± 2</td>
<td>8</td>
<td>55 - 64</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>20 ± 2</td>
<td>4</td>
<td>78 - 82</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>20 ± 2</td>
<td>2</td>
<td>89 - 91</td>
<td>2</td>
</tr>
</tbody>
</table>

It is clear from the above modelling that methods based on the dilution in the plant of 15N labelled fertilizer by N derived from atmosphere are potentially accurate methods to quantify N₂ fixation when large proportion (> 70%) of the N in the fixing crop is derived from atmosphere. However, at lower N₂ fixation level (< 30%) the methodology is much less accurate and under that particular conditions the selection of a standard crop and the stability of the 15N/14N ratio of the substrate is particularly important.
Exercise 1

Calculate % Ndfa in the following three fixing crops (F₁-₃) using four different reference crops (R₁-₄).

<table>
<thead>
<tr>
<th>Crop</th>
<th>% ¹⁵N a.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁</td>
<td>0.15</td>
</tr>
<tr>
<td>F₂</td>
<td>0.04</td>
</tr>
<tr>
<td>F₃</td>
<td>0.23</td>
</tr>
<tr>
<td>R₁</td>
<td>0.27</td>
</tr>
<tr>
<td>R₂</td>
<td>0.26</td>
</tr>
<tr>
<td>R₃</td>
<td>0.22</td>
</tr>
<tr>
<td>R₄</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Exercise 2

Calculate % Ndfa and amount of N fixed of broadbean (F) at each N level using barley (NF) as a reference crop. The experiment was performed at the Seibersdorf Laboratory in 1985.

<table>
<thead>
<tr>
<th>N fert. rate</th>
<th>Crop</th>
<th>Total N kg/ha</th>
<th>% Ndfa</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>F</td>
<td>186.4</td>
<td>0.92</td>
</tr>
<tr>
<td>20</td>
<td>NF</td>
<td></td>
<td>6.21</td>
</tr>
<tr>
<td>100</td>
<td>F</td>
<td>231.7</td>
<td>6.91</td>
</tr>
<tr>
<td>100</td>
<td>NF</td>
<td></td>
<td>33.04</td>
</tr>
<tr>
<td>200</td>
<td>F</td>
<td>201.0</td>
<td>19.06</td>
</tr>
<tr>
<td>200</td>
<td>NF</td>
<td></td>
<td>48.61</td>
</tr>
</tbody>
</table>

Method (2) The A-Value Method (AV)

Often, it is necessary to apply different doses of N to fixing and non-fixing plants. As high levels of inorganic N can depress N₂ fixation it is necessary to apply low amounts of labelled N fertilizer to the fixing crop in order to estimate N₂ fixed. However, such amounts may be too low to support the proper growth of the reference plants, especially in soils of low fertility. For these reasons it is practical to give a reasonable dose of ¹⁵N labelled fertilizer (40-80 kg N/ha) to the reference crop, while the
fixing crop receives a low quantity (10-20 kg N/ha) (Fried and Broeshart, 1975).

When different fertilizer rate is applied to the F and NF crops n is the relative amount of fertilizer applied i.e. \( n = \frac{\text{amount of fertilizer applied to the F crop}}{\text{amount of fertilizer applied to the NF crop}} \).

The assumption (equation 2) which was previously presented is also used for this methodology but \( \% \text{Ndff}_{NF} \), which is the estimated \( \% \text{Ndff} \) at the rate of the F crop has to be calculated in the following equation:

\[
\frac{n \% \text{Ndff}_{NF}}{\% \text{Ndff}_{NF}} = \frac{\% \text{Ndff}}{\% \text{Ndff}_{F}}
\]

(11)

Example 3

In a field experiment two different rates of \(^{15}\text{N}\) labelled ammonium sulphate were applied to F and NF crops i.e. 20 kg N/ha of 5.6 \(^{15}\text{N}\) atom excess to the F crop and 60 kg N/ha of 2.5 \(^{15}\text{N}\) atom excess to the NF crop. What were the \( \% \text{Ndfa} \) for the F crop?

Calculation:

<table>
<thead>
<tr>
<th>Fert. rate</th>
<th>% N-15 a.e. (fert.)</th>
<th>% N-15 a.e. (plant)</th>
<th>% Ndff</th>
<th>% Ndfs</th>
<th>% Ndff</th>
<th>% Ndfa</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF</td>
<td>60</td>
<td>2.50</td>
<td>0.40</td>
<td>16</td>
<td>84</td>
<td>0.063*</td>
</tr>
<tr>
<td>F</td>
<td>20</td>
<td>5.60</td>
<td>0.08</td>
<td>1.4</td>
<td>22.1**</td>
<td>0.063*</td>
</tr>
</tbody>
</table>

\[
\frac{n \times \% \text{Ndff}_{F}}{\% \text{Ndff}_{NF}} = \frac{0.33 \times 16}{84} = 0.063 = \frac{\% \text{Ndff}_{F}}{\% \text{Ndff}_{F}}
\]

** \( \% \text{Ndfs}_{F} = \frac{1.4}{0.063} = 22.1 \% \)

*** \( \% \text{Ndfa} = 100 - \% \text{Ndff}_{F} - \% \text{Ndfs}_{F} \)

\( \% \text{Ndfa} = 100 - 1.4 - 22.1 = 76.5\% \)
Example 3 can also be calculated using the following equation:

\[
\% \text{ Nd} \, \% \text{ Pf} = 100 \left( 1 - \frac{\% \text{ Nd} \, \% \text{ Pf}}{\% \text{ Nd} \, \% \text{ Pf} \left( \frac{1}{n} - 1 \right)} \right) \quad (12)
\]

\[
\% \text{ Nd} = 100 \left[ 1 - \frac{1.4}{0.33 \times 16} \right] + 1.4 \left( \frac{1}{0.33} - 1 \right) = 76.5\%
\]

Equation (8) of Method 1 is a particular case of (12) when \( n \) is equal to 1.

Equation (12) is derived as shown below from (3), (4) and (11).

As previously equation (4) can be written:

\[
e = 100 - c - d
\]

From (11)

\[
d = \frac{b \, c}{a \, n}
\]

and from (3)

\[
d = \frac{c}{a \, n} (100 - a)
\]

or

\[
d = \frac{100 \, c}{a \, n} - \frac{c}{n}
\]

(13a)

Introducing (13a) into (4)

\[
e = 100 - c - \left( \frac{100 \, c}{a \, n} - \frac{c}{n} \right)
\]

or

\[
e = 100 \left( 1 - \frac{c}{n \, a} \right) + c \left( \frac{1}{n} - 1 \right)
\]

or

\[
\% \text{ Nd} = 100 \left( 1 - \frac{\% \text{ Nd} \, \% \text{ Pf}}{n \% \text{ Nd} \, \% \text{ Pf} \left( \frac{1}{n} - 1 \right)} \right) + \% \text{ Nd} \, \% \text{ Pf} \left( \frac{1}{n} - 1 \right)
\]

which is again equation (12).

The amount of fixed N can be calculated according to equation (10).

The use of these formulae is shown in Tables 6 and 7.
Table 6: The following data were recorded for *Vicia faba* (F) and barley (NF) at the Seibersdorf Laboratory, Austria. 20 kg N/ha of $^{15}$N labelled fertilizer was applied to the *Vicia faba* and 100 kg N/ha to the barley. Only one replicate from five is shown in this example.

<table>
<thead>
<tr>
<th>Fert. rate</th>
<th>Total N yield</th>
<th>Ndff</th>
<th>Ndfa</th>
<th>Fixed N</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg N/ha</td>
<td>(kg/ha)</td>
<td>(%)</td>
<td>(%)</td>
<td>(kg/ha)</td>
</tr>
<tr>
<td>F</td>
<td>20 *</td>
<td>151.7</td>
<td>0.877</td>
<td>79</td>
</tr>
<tr>
<td>NF</td>
<td>100 +</td>
<td>18.17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 5.64 % $^{15}$N atom excess;
+ 1.00 % $^{15}$N atom excess;
4-9 see Table 7.

Table 7: Measurements which were made as well as the formulae used to calculate % Ndfa and amount of fixed N in Table 6.

**Measured values:**

1-3) Same as in Table 4

**Calculated values:**

4-7) Same as in Table 4

8) \[ \% \text{Ndfa} = 100 \left( 1 - \frac{\% \text{Ndff}_F}{n \% \text{Ndff}_F + \% \text{Ndff}_F} \right) + \% \text{Ndff}_F \left( \frac{1}{n} - 1 \right) \]

9) \[ N \text{ fixed (kg/ha)} = \frac{\% \text{Ndfa}}{100} \times \text{total N in fixing crop} \]
This method as originally presented used the following A-value concept of Fried and Broeshart (1975):

\[
\frac{\% \text{Ndff}}{A_f} = \frac{\% \text{Ndfs}}{A_s} = \frac{\% \text{Ndfs} + \% \text{Ndfa}}{A_s + a} = \frac{\% \text{Ndfa}}{A_a}
\]

(13b)

where \(A_f\) is the amount of fertilizer applied, whereas \(A_s\) and \(A_a\) are nitrogen available from soil and air, respectively, as expressed in fertilizer units.

For the \(N^*\) reference crop, which derives its nitrogen from only soil and fertilizer, equation (13b) may be rewritten as:

\[
\frac{\% \text{Ndff}}{A_f} = \frac{\% \text{Ndfs}}{A_s}
\]

(14)

From equation (3)

\[
\% \text{Ndfs} = 100 - \% \text{Ndff}
\]

Equation (14) can therefore be re-written as:

\[
A_s = \frac{100 - \% \text{Ndff}}{\% \text{Ndff}_F} \times A_f
\]

(15)

Since the \% Ndff is determined experimentally by the use of \(^{15}\)N, and the amount of fertilizer added \((A_f)\) is known, the available amount of soil \(N\) \((A_s)\) in added fertilizer units can be determined.

However, for the fixing plant, a third source of nutrient, i.e. \(N_2\) is available for plant growth, and therefore

\[
\frac{\% \text{Ndff}}{A_f} = \frac{\% \text{Ndfs} + \% \text{Ndfa}}{A_s + a}
\]

(16)

From equation (4)

\[
\% \text{Ndfs} + \% \text{Ndfa} = 100 - \% \text{Ndff}
\]

Equation (16) can be written as

\[
A_s + a = \frac{100 - \% \text{Ndff}}{\% \text{Ndff}_F} \times A_f
\]

(17)
Again, $\% \text{ Ndff}$ is determined experimentally, and $A_f$ is known. Equations (16) and (17) can then be used to establish $A_a$ as follows:

$$A_a = (A_s + a) - A_s$$  \hspace{1cm} (18)

And from equation (13),

$$\% \text{ Ndfa} = A_a \times \frac{\% \text{ Ndff}}{A_f}$$

The amount of fixed N can be calculated according to equation (10).

Example 4

Using the same data as in example 3 calculate $\% \text{ Ndfa}$ by use of the $A$-value concept.

Calculation:

<table>
<thead>
<tr>
<th>Fert. (fert.)</th>
<th>$% \text{ N-15 a.e.}$ (plant)</th>
<th>$% \text{ N-15 a.e.}$ (plant)</th>
<th>$% \text{ Ndff}$</th>
<th>$A_s$</th>
<th>$A_{s+a}$</th>
<th>$A_a$</th>
<th>$% \text{ Ndfa}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF 60</td>
<td>2.50</td>
<td>0.40</td>
<td>16</td>
<td>315</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F 20</td>
<td>5.60</td>
<td>0.08</td>
<td>1.4</td>
<td>1408</td>
<td>1093</td>
<td>76.5</td>
<td></td>
</tr>
</tbody>
</table>

Calculations in example 3 and 4 give exactly the same $\% \text{ Ndfa}$ or 76.5%. 

150
Exercise 3

Calculate % Ndff and amount of N₂ fixed (kg N/ha) of soybean cv. Chippewa grown at the IAEA Laboratory and inoculated with the following strains of *Bradyrhizobium*. The data is mean of four replicates. (From Hardarson, *et al.*, 1984).

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>N fert. rate (kg/ha)</th>
<th>N yield (kg/ha)</th>
<th>% Ndff</th>
<th>Dry matter yield (ton/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>61 A 23</td>
<td>20</td>
<td>256</td>
<td>2.43</td>
<td>11.4</td>
</tr>
<tr>
<td>61 A 76</td>
<td>20</td>
<td>20</td>
<td>2.92</td>
<td>9.7</td>
</tr>
<tr>
<td>61 A 89</td>
<td>20</td>
<td>288</td>
<td>1.73</td>
<td>11.2</td>
</tr>
<tr>
<td>61 A 117</td>
<td>20</td>
<td>184</td>
<td>2.93</td>
<td>9.9</td>
</tr>
<tr>
<td>61 A 118</td>
<td>20</td>
<td>226</td>
<td>1.56</td>
<td>8.9</td>
</tr>
<tr>
<td>61 A 136</td>
<td>20</td>
<td>254</td>
<td>1.87</td>
<td>10.4</td>
</tr>
<tr>
<td>61 A 148</td>
<td>20</td>
<td>270</td>
<td>2.00</td>
<td>11.3</td>
</tr>
<tr>
<td>61 A 150</td>
<td>20</td>
<td>289</td>
<td>1.43</td>
<td>10.4</td>
</tr>
<tr>
<td>61 A 151</td>
<td>20</td>
<td>295</td>
<td>2.80</td>
<td>10.1</td>
</tr>
<tr>
<td>61 A 152</td>
<td>20</td>
<td>261</td>
<td>1.66</td>
<td>9.2</td>
</tr>
<tr>
<td>Non-nod</td>
<td>100</td>
<td>114</td>
<td>22.7</td>
<td>9.4</td>
</tr>
</tbody>
</table>
Exercise 4

Calculate % Ndfa and amount of N\textsubscript{2} fixed in broad bean (B) which was harvested at different times (T\textsubscript{1-7}) during the growing season. Wheat (W) was used as a reference crop. 20 kg/ha of \textsuperscript{15}N labelled fertilizer/ha was applied to broad bean and 100 kg N/ha to the wheat. (From Zapata et al., 1987).

<table>
<thead>
<tr>
<th>Time</th>
<th>Crop</th>
<th>N yield kg/ha</th>
<th>% Ndfa</th>
</tr>
</thead>
<tbody>
<tr>
<td>T\textsubscript{1}</td>
<td>B</td>
<td>12</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td></td>
<td>37.3</td>
</tr>
<tr>
<td>T\textsubscript{2}</td>
<td>B</td>
<td>55</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td></td>
<td>37.7</td>
</tr>
<tr>
<td>T\textsubscript{3}</td>
<td>B</td>
<td>79</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td></td>
<td>37.1</td>
</tr>
<tr>
<td>T\textsubscript{4}</td>
<td>B</td>
<td>111</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td></td>
<td>32.7</td>
</tr>
<tr>
<td>T\textsubscript{5}</td>
<td>B</td>
<td>137</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td></td>
<td>31.6</td>
</tr>
<tr>
<td>T\textsubscript{6}</td>
<td>B</td>
<td>210</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td></td>
<td>27.2</td>
</tr>
<tr>
<td>T\textsubscript{7}</td>
<td>B</td>
<td>209</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td></td>
<td>27.6</td>
</tr>
</tbody>
</table>

T\textsubscript{1}: 56 days after planting, mid-vegetative growth
T\textsubscript{2}: 77 " " " , first flower
T\textsubscript{3}: 84 " " " , 50% flowering
T\textsubscript{4}: 91 " " " , beginning of pod set
T\textsubscript{5}: 98 " " " , 50% pod set
T\textsubscript{6}: 105 " " " , early pod filling
T\textsubscript{7}: 112 " " " , mid-pod filling
T\textsubscript{8}: 126 " " " , maturity
Method (3) Single treatment method (ST)

This involves a combination of two isotope methods (Fried and Broeshart, 1981), i.e., the A-value method described previously and the "single treatment" experiment without interaction (Fried et al., 1975).

In the "single treatment" experiment all treatments are the same as far as the plant and soil are concerned but only one fertilizer application in each treatment combination has been labelled with isotopes as shown in the following illustration:

<table>
<thead>
<tr>
<th>Crop</th>
<th>Plot</th>
<th>Amount of N applied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10*</td>
</tr>
<tr>
<td>F</td>
<td>a</td>
<td>10</td>
</tr>
<tr>
<td>F</td>
<td>b</td>
<td>20*</td>
</tr>
<tr>
<td>NF</td>
<td>a</td>
<td>10</td>
</tr>
<tr>
<td>NF</td>
<td>b</td>
<td>20</td>
</tr>
</tbody>
</table>

Therefore, nitrogen fixation is identical in the matching treatments a and b within the limit of experimental error. By this approach, time of application of fertilizer, fertilizer source, and placements at the same time as \( \text{N}_2 \) fixation can be evaluated and inserted as treatments A and B in the above illustration.

The calculations for this type of experiments are similar to the ones for Method 2, where different amounts of labelled fertilizer are applied to the fixing and the non-fixing crops. However, correction has to be made for the proportion of nitrogen taken up from the other unlabelled fertilizer applied. The same assumption is used as for the previous methods.

The following equations show the sources of nitrogen for the fixing and the non-fixing crops:

\[
\text{Non-fixing crop: } \% \text{Ndff}_{\text{NF}} + \% \text{Ndfs}_{\text{NF}} + \% \text{Nduf}_{\text{NF}} = 100
\]

or

\[
a + b + g = 100 \quad (19)
\]
where % Ndff* and % Ndfuf is percentage N derived from labelled and unlabelled fertilizer, respectively.

\[
\text{Fixing crop: } \% \text{Ndff}^* + \% \text{Ndfs}^* + \% \text{Ndfa} + \% \text{Ndfuf} = 100 \\
\text{c + d + e + f} = 100 \quad (20)
\]

An equation to quantify N\textsubscript{2} fixation can be derived from equations (19) and (20) and the following one which is the same as (11) in Method 2.

\[
\frac{a}{b} = \frac{c}{d}
\]

From which

\[
d = \frac{bc}{an}
\]

and from (19)

\[
d = \frac{c}{an} (100 - a-g)
\]

or

\[
d = \frac{100c}{an} - \frac{c}{n} - \frac{cg}{an} \quad (21)
\]

Introducing (21) into (20)

\[
e = 100 - c - \left(\frac{100c}{an} - \frac{c}{n} - \frac{cg}{an}\right) - f
\]

or

\[
e = 100 - c - \frac{100c}{an} + \frac{c}{n} + \frac{cg}{an} - f
\]

or

\[
e = 100 \left(1 - \frac{c}{na}\right) + c \left(\frac{1}{n} - 1 + \frac{g}{an}\right) - f \quad (22)
\]

Equation (22) becomes like (12) when g and f are 0.

The amount of fixed N can be calculated according to equation (10). The use of these formulae is shown in Tables 8 and 9.
Table 8: The following data were recorded at the Seibersdorf Laboratory, Austria in 1980 for Vicia faba (fixing) and wheat (non-fixing) using "single treatment" experimental approach. (Data from Fried and Broeshart, 1981).

<table>
<thead>
<tr>
<th>N Applied (kg/ha)</th>
<th>Total N yield (kg/ha)</th>
<th>Ndff (%)</th>
<th>Ndfa (%)</th>
<th>Fixed N (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vicia faba</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a broadcast</td>
<td>10*</td>
<td>10</td>
<td>243.3</td>
<td>1.3 70 170</td>
</tr>
<tr>
<td>1b banded</td>
<td>10</td>
<td>10*</td>
<td>225.4</td>
<td>1.9 70 158</td>
</tr>
<tr>
<td><strong>Wheat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a broadcast</td>
<td>20*</td>
<td>20</td>
<td>243.3</td>
<td>1.3 70 170</td>
</tr>
<tr>
<td>2b banded</td>
<td>20</td>
<td>20*</td>
<td>225.4</td>
<td>1.9 70 158</td>
</tr>
</tbody>
</table>

*: labelled with $^{15}$N

4-9) See Table 9)

Table 9: Measurement which were made and formulae used to calculate values in Table 8.

**Measure values:**

1-3) Same as in Table 4

**Calculated values:**

4-7) Same as in Table 4

8) $$e = 100 \left(1 - \frac{c}{n a}\right) + c \left(\frac{1}{n} - 1 + \frac{g}{n a}\right) - f$$

$$e : \% \text{ Ndfa}$$

$$a : \% \text{ Ndff}_{NP}$$

$$c : \% \text{ Ndff}_{F}$$

$$g : \% \text{ Nduf}_{NP}$$

$$f : \% \text{ Nduf}_{F}$$

9) $$N \text{ fixed (kg/ha)} = \frac{\% \text{ Ndfa} \times \text{ total N in fixing crop}}{100}$$
Example 5

The following examples show the experimental treatments to study N fertilizer practices for legume crops.

Situation 1:

As shown previously N₂ fixation can easily be calculated when only starter N is applied:

<table>
<thead>
<tr>
<th>Starter N (kg N/ha)</th>
<th>% Ndff</th>
</tr>
</thead>
<tbody>
<tr>
<td>F 20</td>
<td>4</td>
</tr>
<tr>
<td>NF 80</td>
<td>27</td>
</tr>
</tbody>
</table>

Calculation:

\[
\% \text{ Ndfa} = 100 \left(1 - \frac{\% \text{ Ndff}_F}{n \% \text{ Ndff}_{NF}}\right) + \% \text{ Ndff}_F \left(\frac{1}{n} - 1\right)
\]

\[
\% \text{ Ndfa} = 100 \left(1 - \frac{4}{0.25 \times 27}\right) + 4 \left(\frac{1}{0.25} - 1\right)
\]

\[
\% \text{ Ndfa} = 52.7\%
\]

Situation 2:

The following set of treatment are needed to determine the effect of two fertilizer N practices on symbiotic nitrogen fixation without interaction:

<table>
<thead>
<tr>
<th>Fertilizer application</th>
<th>N yield (kg N/ha)</th>
<th>% Ndff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starter</td>
<td>Foliar</td>
<td></td>
</tr>
<tr>
<td>F a 20*</td>
<td>80</td>
<td>168</td>
</tr>
<tr>
<td>F b 20</td>
<td>80*</td>
<td>178</td>
</tr>
<tr>
<td>NF 80*</td>
<td>0</td>
<td>56</td>
</tr>
</tbody>
</table>
Calculation of % Ndfa:

\[
\% \text{Ndfa} = 100 \left( 1 - \frac{\% \text{Ndff}_F}{n \% \text{Ndff}_NF} \right) \cdot \% \text{Ndff}_F \left( \frac{1}{n} - 1 + \frac{\% \text{Ndff}_NF}{n \% \text{Ndff}_NF} \right) - \% \text{Ndff}_F
\]

for plot a

\[
\% \text{Ndfa} = 100 \left( 1 - \frac{3}{0.25 \times 27} \right) + 3 \left( \frac{1}{0.25} - 1 + \frac{0}{0.25 \times 27} \right) - 18
\]

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%

Thus for F:

\%
For expressing the level of natural $^{15}\text{N}$ abundance the more sensitive expression of $\delta^{15}\text{N} \%$ is often used (Vose, 1982, Axmann and Zapata, this volume).

$$\delta^{15}\text{N} \% = \frac{^{15}\text{N} / ^{14}\text{N} \text{ sample}}{^{15}\text{N} / ^{14}\text{N} \text{ standard}} - 1 \times 100 \tag{23}$$

If $N_2$ fixing plants are grown in soil which has a higher $\delta^{15}\text{N}$ value than the atmosphere then $\% N_{dfa}$ can be quantified according to the following equation:

$$\% N_{dfa} = (1 - \frac{^{15}\text{N} \% \text{ (fixing crop)}}{^{15}\text{N} \% \text{ (non-fixing crop)}} \times \frac{^{15}\text{N} \% \text{ air}}{^{15}\text{N} \% \text{ air}}) \times 100 \tag{24}$$

The main disadvantages of this method are the rather small differences in $^{15}\text{N}$ abundance being traced, the high variability of $^{15}\text{N}$ abundance in soils, which therefore raises doubts as to the suitability of the method to estimate $N_2$ fixation. It may therefore only serve as a semi-quantitative estimate of $N_2$ fixation.

REFERENCES


BURRES, R.H., MILLER, C.E. Application of $^{15}\text{N}$ to the study of biological nitrogen fixation. Science 93 (1941) 114-115.


TECHNIQUES IN STUDIES OF PHOTOSYNTHESIS

K.S. KUMARASINGHE
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,
International Atomic Energy Agency,
Vienna

INTRODUCTION

Agriculture is primarily a system whereby the solar energy is exploited for human consumption through photosynthesis. As the primary source of energy, photosynthesis provides the energy for food, feed, fuel and fibre. The yields of crop plants depend mainly on the efficiency of the photosynthetic process and this in turn is determined by genetic, environmental and management factors. The genetic characteristics of the plant determine the potential maximum rate of photosynthesis, the rate of dry matter production and the form and nature of storage organs, such as those usually harvested as food. The environmental factors such as water availability, temperature, day-length, light intensity and availability of nutrients determine to what extent this potential can be reached.

The use of both stable and radioactive isotopes has led to major advances in the understanding of the basic mechanisms of photosynthesis. An early use of isotopic material in photosynthetic investigations was the demonstration using $^{18}$O, that $O_2$ evolved in photosynthesis was derived from water rather than from $CO_2$. When the long-lived isotope of carbon, $^{14}$C, became available in 1945, its use, coupled with two-dimensional chromatography developed a few years earlier, enabled Calvin and Benson (1948) to devise experiments to elucidate the pathway of photosynthetic $^{14}$CO$_2$ fixation.

THE PHOTOSYNTHETIC PROCESS

The photosynthetic process takes place in the chloroplasts. These are lens-shaped organelles, 1 to 10 $\mu$m in diameter and displaying two key areas: (1) the lamellae (membranes), consisting of stroma lamellae (single lamella) and grana lamellae (stacked lamellae), both of which contain the photosynthetic pigment, the chlorophylls, and (2) the stroma, a granular fluid matrix where the reduction of $CO_2$ occurs. The transformation of the energy of sunlight into chemical energy (photophosphorylation) occurs in lamellae. The photochemical events of the light phase result in (i) the production of a strong
reducing agent, reduced nicotinamide adenine dinucleotide phosphate (NADPH), (ii) the accompanying evolution of \( \text{O}_2 \) as a by-product of the splitting of water and (iii) the formation of ATP which is coupled to the flow of electrons from \( \text{H}_2\text{O} \) to NADP. Both NADPH and ATP are needed to convert \( \text{CO}_2 \) to organic molecules.

![Diagram showing the process of photosynthesis](image)

Photophosphorylation (The light reactions)  

\[
\begin{align*}
\text{H}_2\text{O} & \longrightarrow \text{ADP} + \text{Pi} \\
\text{O}_2 & \longrightarrow \text{NADP} \\
\text{ATP} & \longrightarrow \text{NADPH} \\
\text{CO}_2 & \longrightarrow (\text{CH}_2\text{O})_n
\end{align*}
\]

\( \text{CO}_2 \) Fixation (The dark reactions)

**THE CARBON DIOXIDE FIXATION PATHWAYS**

On the basis of the first stable product of photosynthetic \( \text{CO}_2 \) fixation, plants can now be divided into two main groups, i.e. the C-3 and C-4 plants. A third group namely CAM (Crassulacean Acid Metabolism) plants is a variation of C-4 metabolism (Zelitch, 1971).

**C-3 photosynthesis**

This pathway of \( \text{CO}_2 \) fixation can be separated into four major phases (Hall and Rao, 1987):

(i) Carboxylation phase

In this first phase the five carbon sugar ribulose bisphosphate (RuBP) is carboxylated to form two molecules of phosphoglyceric acid (PGA). Since PGA is the first stable product of this metabolic sequence and is a 3-carbon compound, the pathway is called the C-3 pathway. It is also often referred to as the Calvin cycle (Fig. 1) after its elucidation by Calvin and his co-workers. This primary carboxylation reaction is catalysed by the enzyme ribulose bisphosphate carboxylase/oxygenase (RUBISCO).
(ii) Reduction phase

During this phase, the PGA formed in the carboxylation phase is reduced to 3-carbon sugar level (Triose-P) using the assimilatory power of NADPH and ATP generated in photochemical reactions.

(iii) Product synthesis phase

Part of the triose-P molecules formed in phase (ii) is finally converted to the end products of photosynthesis usually sucrose or starch. But, other compounds such as fats, fatty acids, amino acids, organic acids and other carbohydrates can also be synthesized depending on the plant species and the environmental conditions.

(iv) Regeneration phase

This phase ensures that the CO$_2$ acceptor RuBP is regenerated in a cyclic manner. Some of the triose-P molecules, through a complex series of reactions, and utilizing some of the ATP generated in photochemical reactions finally replace the RuBP which can again undergo further carboxylation.

C-4 photosynthesis

From about 1946 to 1966 the Calvin cycle was considered the only pathway for CO$_2$ fixation in higher plants. Then, in 1966 Hatch and Slack working with sugarcane presented detailed evidence that another pathway (Fig. 2) for
$\text{CO}_2$ fixation exists in some species. In this pathway the initial carboxylation reaction is catalysed by an enzyme called phosphoenolpyruvate (PEP) carboxylase in which the $\text{CO}_2$ acceptor phosphoenolpyruvate is carboxylated to form a four carbon compound oxaloacetate. The oxaloacetate is rapidly converted to malate or aspartate, or both. These are then translocated to bundle sheath cells around the vascular bundle where they are converted to pyruvate. In the conversion to pyruvate a molecule of $\text{CO}_2$ is released which is again trapped by RuBP to form 3-PGA, in the Calvin cycle, which in these plants is operative in the bundle sheath cells. Species with this mode of $\text{CO}_2$ fixation are called C-4 plants because their first product of photosynthesis (oxaloacetate) is a four carbon molecule. Some major physiological, biochemical and anatomical differences (Table 1) exist, between C-3 and C-4 plants which leads to marked differences in crop productivity between them (Coombs, 1984).

In C-4 plants a distinct bundle sheath layer develops around the vascular tissue. These bundle sheath cells are rich in organelles, particularly large and dark green chloroplasts that often show reduced grana development. The chloroplasts synthesize large amounts of starch. Chloroplasts are also present in the mesophyll cells of C-4 plants, and these mesophyll cells form a concentric ring around the bundle sheath layer. This arrangement of chlorenchyma layers is termed the 'Kranz syndrome'. In C-3 plants a bundle sheath layer is either absent or when present as 'in the case of some C-3 grasses, no chloroplasts are present and as a result the entire photosynthetic machinery occurs in mesophyll cells.
<table>
<thead>
<tr>
<th>Criterion</th>
<th>C-3</th>
<th>C-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. First stable product of photosynthesis</td>
<td>PGA</td>
<td>Malate/Aspartate</td>
</tr>
<tr>
<td>2. Primary CO₂ acceptor</td>
<td>RuBP</td>
<td>PEP</td>
</tr>
<tr>
<td>3. Photosynthetic rate (mg CO₂/dm²/h)</td>
<td>15-30</td>
<td>30-80</td>
</tr>
<tr>
<td>4. Temperature optima for photosynthesis</td>
<td>Approx. 20°C</td>
<td>Approx. 30°C</td>
</tr>
<tr>
<td>5. Effect of O₂ on photosynthesis</td>
<td>Markedly inhibited</td>
<td>Insensitive</td>
</tr>
<tr>
<td>6. CO₂ compensation point</td>
<td>-50 ppm</td>
<td>0-10 ppm</td>
</tr>
<tr>
<td>7. Leaf anatomy</td>
<td>Bundle sheath cells either absent or if present contain no chloroplasts.</td>
<td>Bundle sheath cells containing chloroplasts are always present</td>
</tr>
<tr>
<td>8. Presence of starch</td>
<td>Present in mesophyll cells</td>
<td>Normally present in bundle sheath cells</td>
</tr>
<tr>
<td>9. Photorespiration</td>
<td>High</td>
<td>Low or absent</td>
</tr>
<tr>
<td>10. Average annual yield/ha</td>
<td>15-30 t.</td>
<td>65-80 t.</td>
</tr>
<tr>
<td>11. Maximum growth rate (g dry wt./m²/day)</td>
<td>20-35</td>
<td>50-60</td>
</tr>
<tr>
<td>12. Representative plant species</td>
<td>Wheat, rice, barley legumes, most fruits and vegetables, etc.</td>
<td>Maize, sorghum, sugar-cane, many other grasses and sedges, Amaranthus, Portulaca, etc.</td>
</tr>
</tbody>
</table>

**Crassulacean Acid Metabolism (CAM)**

CAM plants are the third group of plants which show distinct metabolic characteristics. These species which comprise primarily of arid-zone plants also have a C-4 cycle, but the movement of carbon is separated in time rather than in space. In many CAM plants the metabolic variation is an adaptation to arid environmental conditions. Carbon dioxide is fixed by the PEP carboxylase.
reaction in the dark (stomata open) and accumulates as malate in the leaf cell vacuoles. In the light, the stomata close thereby conserving the little water that is available, while CO₂ is released internally from the malate and refixed through the Calvin cycle (Osmond and Holtum 1981). Thus CAM plants have developed an ingenious physiological and biochemical method of reducing water loss and escaping drought. They often are important crop plants (e.g. Pineapple, Agave and Prickly pear) growing in arid and semi-arid habitats.

**Photorespiration**

Photorespiration involves the evaluation of CO₂ in light by photosynthesizing tissues, using early products of photosynthesis as substrate (Tolbert, 1971). This substrate is now known to be the two carbon glycollate. The CO₂ and O₂ in the atmosphere compete for the enzyme RUBISCO; high CO₂ and low O₂ promote carboxylation and therefore the formation of Calvin cycle products while high O₂ and low CO₂ promote oxygenation and therefore photorespiration. As a result of the dual properties of this enzyme, photorespiration becomes an inevitable consequence of C-3 photosynthesis under normal atmospheric conditions. During the operation of the photorespiratory pathway (Fig. 3) four molecules of glycollate are ultimately converted to one molecule of hexose, and two molecules of CO₂ are released as a by-product. ATP is also synthesized during this reaction. Various assay methods have shown that at high irradiance, the release of CO₂ by photorespiration amounts to 25-50% of net

![FIG. 3. The photorespiratory pathway.](image-url)
photosynthesis (Kumarasinghe et al., 1977). Photorespiration, however, is undetectable or operates at an insignificant level in C-4 species which probably accounts for the high net photosynthetic rates in C-4 plants. The pathway is complex and involves three types of cell organelles - the chloroplasts, mitochondria and the peroxisomes. Within the cell these bodies lie in close proximity which facilitates the flow of carbon between them. The general consensus of opinion is that photorespiration is a wasteful process plants could do without. However, attempts to reduce photorespiration and thereby increase photosynthesis and crop production under field conditions, particularly using chemicals or by selecting for photorespiratory deficient mutants have not met with much success so far.

NITROGEN FIXATION IN RELATION TO PHOTOSYNTHESIS

Certain micro-organisms, mainly bacteria and blue-green algae are capable of reducing atmospheric nitrogen to ammonia, a process referred to as biological nitrogen fixation (see Hardarson and Danso, this volume). The organisms may be free-living, or symbiotic as in the case of legume-rhizobia or Azolla-Anabaena associations. The basic requirements for biological nitrogen fixation are,
1. The enzyme complex nitrogenase
2. A strong reducing agent of low redox potential (reduced ferredoxin, flavodoxin, NAD or NADP)
3. ATP

The enzyme nitrogenase catalyses the reduction of $N_2$ to $NH_3$.

$$ N_2 + 3XH_2 + 6ATP \rightarrow 2NH_3 + 3X + 6ADP + Pi $$

The reductant ($XH_2$) and the ATP required for the nitrogenase system are provided by the products of photosynthesis. Further, a supply of photosynthates is also required to provide the carbon skeletons necessary to convert $NH_3$ to complex nitrogenous compounds of the cell.

The importance of photosynthates to nitrogen fixation has been clearly demonstrated by a number of workers (Lawrie and Wheeler, 1975; Pate et al., 1979). In legumes, during the early vegetative phase, about 60 percent of the daily acquired photosynthate is transported down the stem, about 40 percent being diverted to the roots and 30 percent to the nodules. Approximately half of this carbon input to the nodules is returned to the shoots as fixation products and the rest is used for respiration and growth of the nodule.
It has been shown that CO₂ enrichment increased nitrogen fixation by improving photosynthesis of plants, growing in enclosed jars. In soybeans, for instance, the increase in CO₂ concentration in air from 300 to 1200 ppm resulted in a four to five-fold increase in nitrogen fixation. This was due to the formation of a greater number of nodules, a higher efficiency of the nodules and a prolongation of the active period of nitrogen fixation. In subsequent experiments, it was found that lowering the O₂ concentration in the ambient atmosphere has almost the same effect on nitrogen fixation as increasing CO₂ concentration. The conclusion was drawn that both increasing CO₂ and lowering O₂ reduced photorespiration and thus more photosynthates were available for nitrogen fixation. In many legumes the amount of downward transport of carbon decreases with age, particularly during the reproductive phase when the pods and seeds compete successfully for carbon and nitrogen with other plant organs. It therefore seems important to maintain a steady supply of photosynthates to the nodules so that nitrogen fixation can be maintained during the critical stage of heavy demand for nitrogen.

PRACTICAL EXERCISES

14 Carbon technique

Exercise No. 1

Objective

1. To familiarize with the technique of preparing \(^{14}\)CO₂ into gas cylinders.
2. To gain practical experience in the feeding of \(^{14}\)CO₂ into leaves.
3. To measure the photosynthetic rates of some crop plants using \(^{14}\)CO₂.

Materials and equipment

1. Plant material: C-3 and C-4 plants.
2. Compressed gas cylinder containing \(^{14}\)CO₂ air, in which the absolute CO₂ concentration and the specific activity of \(^{14}\)CO₂ are known.
3. Compressed air cylinder containing ordinary air.
4. Leaf chamber and other accessories.
5. Cork borer in which the area is known.
6. Forceps.
Experimental procedure

Preparation of $^{14}\text{CO}_2$ gas - (Refer to Fig. 4)

1. Evacuate cylinder D using a high vacuum pump and connect to E.
2. Take the precalculated quantity of $^{14}\text{C}$ labelled sodium carbonate and the unlabelled sodium carbonate in A.
3. Using F inject lactic acid (10%) into A. Keep C and D in closed position.
4. After allowing time for the liberation of $^{14}\text{CO}_2$ gently open D so that $\text{CO}_2$-free air passing through B flushes the $^{14}\text{CO}_2$ produced in A, into D.
5. Once atmospheric pressure is attained in D (when bubbling stops in G) close E to A.
6. Open E to C and gradually increase the pressure in D until the desired pressure is reached.
7. Close C and D. Your $^{14}\text{CO}_2$ gas cylinder (D) is now ready for use.

Feeding $^{14}\text{CO}_2$ gas into a leaf

A schematic diagram of the $^{14}\text{CO}_2$ feeding apparatus is shown in Fig. 5.
FIG. 5. Schematic diagram of the field apparatus for exposing leaves to $^{14}$CO$_2$. (A) $^{14}$CO$_2$ gas cylinder; (B) $^{12}$CO$_2$ gas cylinder; (C) polythene tubing; (D) three-way stopcock; (E) flow meter; (F) feeding chamber; (G) soda-lime tower.

1. Open cylinder A and maintain a flow rate of about 400 ml/min in E.
2. Repeat above procedure with cylinder B and close tap D to both A and B.
3. Open leaf chamber F, insert leaf and carefully close so that the chamber is made air tight.
4. Open tap D to cylinder A and allow passage of $^{14}$CO$_2$ through the leaf chamber. Start timing now.
5. After the desired period of exposure of leaf to $^{14}$CO$_2$, close tap D to cylinder A and open to cylinder B containing ordinary air to flush the system of residual $^{14}$CO$_2$ gas. About 1 min. is sufficient for this purpose. Flushing time depends on the length of tubing between D and G, and the flow rate used.
6. Open the leaf chamber F and punch out a disc from the $^{14}$CO$_2$ fed area of the leaf, using the cork borer provided.
7. Plunge the leaf disc into a scintillation vial containing 2 ml of soluene (direct in-vial sample digestion). Allow about 24 hours for complete digestion.
8. The use of the scintillation counter is explained by Buchtela (This volume). The counter gives the number of disintegrations per minute (dpm) after appropriate quench correction. Add 5 ml of Instagel or any other
suitable scintillation cocktail into your sample vial and count the radioactivity.

10. Calculate the net photosynthetic rates of your leaf sample using equation 1, and discuss your results.

An example

In an experiment carried out to measure the photosynthetic rates of some crop plants using $^{14}$CO$_2$ the following results were obtained (Table 2).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Radioactivity in leaf disc dpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>866,678</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>799,384</td>
</tr>
<tr>
<td>Amaranthus</td>
<td>750,076</td>
</tr>
<tr>
<td>Banana</td>
<td>233,156</td>
</tr>
<tr>
<td>Apple</td>
<td>266,464</td>
</tr>
<tr>
<td>Soybean var. 1</td>
<td>466,312</td>
</tr>
<tr>
<td>&quot; var. 2</td>
<td>249,810</td>
</tr>
<tr>
<td>&quot; var. 3</td>
<td>283,118</td>
</tr>
<tr>
<td>&quot; var. 4</td>
<td>266,465</td>
</tr>
</tbody>
</table>

Other measurements made

- Specific activity of $^{14}$CO$_2$ used = 0.5 µCi/µmole
- Area of leaf disc = 0.04 dm$^2$
- Time of exposure to $^{14}$CO$_2$ = 1 min

Calculations

Let, the dpm of leaf sample be $d$
the sp. activity of $^{14}$CO$_2$ gas used = $S$
leaf area = $a$
time of exposure to $^{14}$CO$_2$ = $t$

Now,

$1 \text{ µCi} = 2.2 \times 10^6 \text{ dpm}$
Therefore, the number of \( \mu \text{Ci} \) in \( d = \frac{d}{2.2 \times 10^6} \)

And the number of \( \mu \text{moles} \) \( \text{CO}_2 = \frac{d}{2.2 \times 10^6} \times \frac{1}{S} \)

1 \( \mu \text{mole} \) \( \text{CO}_2 = 44 \mu \text{g} \)

Therefore, \( \frac{d}{2.2 \times 10^6} \times \frac{1}{0.5} \) \( \mu \text{moles} \) \( \text{CO}_2 = \frac{d}{2.2 \times 10^6} \times \frac{1}{S} \times \frac{44}{1} \mu \text{g} \) \( \text{CO}_2 \)

\[
= \frac{d}{2.2 \times 10^6} \times \frac{1}{S} \times \frac{44}{1} \times \frac{1}{1000} \text{ mg CO}_2
\]

This is the amount of \( \text{CO}_2 \) fixed by a leaf area of a \( \text{dm}^2 \) in a time of \( t \) minutes.

Therefore the amount of \( \text{CO}_2 \) fixed per square decimeter (\( \text{dm}^2 \)) per hour is represented by the equation,

\[
\frac{d}{2.2 \times 10^6} \times \frac{1}{S} \times \frac{44}{1} \times \frac{1}{1000} \times \frac{1}{a} \times \frac{60}{t} \text{ mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{h}^{-1}
\]

or

\[
\text{NPR} = \frac{d}{a} \times \frac{60}{S} \times \frac{44}{t} \times \frac{1}{2.2 \times 10^9} \text{ mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{h}^{-1} \quad \text{(Equation 1)}
\]

Where NPR = Net photosynthetic rate (\( \text{mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{h}^{-1} \)).

The photosynthetic rates calculated using equation 1 are given in Table 3.

Table 3. Photosynthetic rates as measured in various plant species

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Net photosynthetic rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(( \text{mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{h}^{-1} ))</td>
</tr>
<tr>
<td>Maize</td>
<td>52</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>48</td>
</tr>
<tr>
<td>Amaranthus</td>
<td>46</td>
</tr>
<tr>
<td>Banana</td>
<td>14</td>
</tr>
<tr>
<td>Apple</td>
<td>16</td>
</tr>
<tr>
<td>Soybean var. 1</td>
<td>28</td>
</tr>
<tr>
<td>Soybean var. 2</td>
<td>15</td>
</tr>
<tr>
<td>Soybean var. 3</td>
<td>17</td>
</tr>
<tr>
<td>Soybean var. 4</td>
<td>16</td>
</tr>
</tbody>
</table>

172
Measurement of carbon distribution by radioactivity counting

Exercise No. 2

Objective

To study the distribution of carbon between leaves, stems, roots and nodules of a legume, following photosynthesis in $^{14}$CO$_2$.

Materials and equipment

1. Plant material: six week old soybean plants in pots.
2. $^{14}$CO$_2$ feeding apparatus (as in exercise 1).
3. Whole plant feeding chamber.
5. Forceps.
6. Disposable gloves.
7. Scintillation vials containing soluene (2 ml).
8. Instagel or any other suitable scintillation cocktail.

Experimental procedure

1. Feed $^{14}$CO$_2$ to a legume plant using a feeding chamber designed to accommodate a whole plant. The other component of the gas flow system are basically the same as in Fig. 5.
2. Remove plants from the feeding chamber and punch out about 4-5 leaf discs to determine the net photosynthetic rate. Record fresh weight of the leaf disc.
3. Leave for 4-5 hours in ordinary air for translocation of photosynthates. The chase period in ordinary air will vary according to the type of experiment.
4. Separate the plants into leaves, stems, roots and nodules. Record the total fresh weight in each component.
5. Chop the component plant material separately into 2-4 mm segments.
6. Remove a sub-sample of about 100 mg into a specimen vial containing 1 ml of tissue solubilizer (Ex. Soluene).
7. After allowing about 24 hours at 60°C for complete digestion of plant material, remove a 100 $\mu$l aliquot into a scintillation vial.
8. Add 10 ml of Instagel or any other suitable scintillation cocktail and count the radioactivity using a scintillation counter.
9. Alternatively, a sample can be dried and a sub-sample of about 50 mg oxidized using an automatic sample oxidizer (Packard or Harvey Instrument Corporation).

An example

The following results were recorded in an experiment carried out to determine the distribution of $^{14}$C in four varieties of soybean.

Table 4: Radioactivity in leaf discs

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Radioactivity (dpm) in leaf discs</th>
<th>NPR mg CO$_2$.dm$^{-2}$.h$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean var. 1</td>
<td>466,312</td>
<td>28</td>
</tr>
<tr>
<td>&quot; var. 2</td>
<td>249,810</td>
<td>15</td>
</tr>
<tr>
<td>&quot; var. 3</td>
<td>283,118</td>
<td>17</td>
</tr>
<tr>
<td>&quot; var. 4</td>
<td>266,465</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 5: Radioactivity in 100 mg plant samples

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Radioactivity (dpm) in 100 mg sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>Soybean var. 1</td>
<td>693,817</td>
</tr>
<tr>
<td>&quot; var. 2</td>
<td>212,691</td>
</tr>
<tr>
<td>&quot; var. 3</td>
<td>291,462</td>
</tr>
<tr>
<td>&quot; var. 4</td>
<td>621,366</td>
</tr>
</tbody>
</table>

Table 6: Total fresh weight (g) of plant parts

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
<th>Nodules</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean var. 1</td>
<td>15,61</td>
<td>12,32</td>
<td>6,91</td>
<td>2,73</td>
<td>37,57</td>
</tr>
<tr>
<td>&quot; var. 2</td>
<td>11,72</td>
<td>10,23</td>
<td>5,42</td>
<td>1,20</td>
<td>28,57</td>
</tr>
<tr>
<td>&quot; var. 3</td>
<td>12,31</td>
<td>10,84</td>
<td>4,82</td>
<td>1,82</td>
<td>29,79</td>
</tr>
<tr>
<td>&quot; var. 4</td>
<td>11,13</td>
<td>9,32</td>
<td>3,61</td>
<td>1,52</td>
<td>25,58</td>
</tr>
</tbody>
</table>
Table 7: Total radioactivity (dpmx10^6) in plant parts

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
<th>Nodules</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean var. 1</td>
<td>108,23</td>
<td>34,93</td>
<td>40,71</td>
<td>9,67</td>
<td>193,54</td>
</tr>
<tr>
<td>var. 2</td>
<td>24,87</td>
<td>12,89</td>
<td>17,34</td>
<td>36,54</td>
<td>91,64</td>
</tr>
<tr>
<td>var. 3</td>
<td>35,86</td>
<td>14,96</td>
<td>20,76</td>
<td>43,69</td>
<td>115,27</td>
</tr>
<tr>
<td>var. 4</td>
<td>68,98</td>
<td>22,64</td>
<td>30,59</td>
<td>10,63</td>
<td>132,84</td>
</tr>
</tbody>
</table>

Table 8: Percent radioactivity in different plant parts

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
<th>Nodules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean var. 1</td>
<td>56</td>
<td>18</td>
<td>21</td>
<td>05</td>
</tr>
<tr>
<td>var. 2</td>
<td>27</td>
<td>14</td>
<td>19</td>
<td>40</td>
</tr>
<tr>
<td>var. 3</td>
<td>31</td>
<td>13</td>
<td>18</td>
<td>38</td>
</tr>
<tr>
<td>var. 4</td>
<td>52</td>
<td>17</td>
<td>23</td>
<td>08</td>
</tr>
</tbody>
</table>

Calculations

1. Using equation 1, calculate the NPR of the leaf discs. The results are shown in Table 1.

2. Calculate the total radioactivity in each plant component using the following equation.

\[
TR = \frac{TFW \times SR}{SFW}
\]  
(Equation 2)

Where,

- \(TR\) = Total radioactivity (dpm) in plant part
- \(SR\) = Radioactivity in sub-sample
- \(TFW\) = Total fresh weight
- \(SFW\) = Fresh weight of sub-sample

The parameters \(SR\), \(SFW\) (Table 5) and \(TFW\) (Table 6) are experimentally determined, and \(TR\) (Table 7) is calculated.
3. To determine the percent distribution of $^{14}$C in different plant parts (Table 8), first add up the total radioactivity (TR) in component plant parts to obtain the radioactivity in the whole plant. Now, using the following equation calculate the percent $^{14}$C in each plant component.

$$\% \text{ C in plant part} = \frac{TR}{WPR} \times 100$$  \hspace{1cm} (Equation 3)

Where,

$TR = \text{Total radioactivity in plant part}$

$WPR = \text{Radioactivity in whole plant}$

**Measurement of carbon distribution by autoradiography**

**Introduction**

Autoradiography is a technique whereby an image or a picture is produced on a photographic plate showing the distribution and concentration of a radioisotope in a given body. Autoradiography is broadly divided into two types, (i) micro-autoradiography and (ii) macro-autoradiography. In macro-autoradiography the presence of the radioisotope is located through images visible to the naked eye whereas in micro-autoradiography images are observed at cellular and sub-cellular level with either light or electron microscopes. In this training manual, only the techniques involved in macro-autoradiography will be dealt with. In general, in macro-autoradiography, relatively large material containing the radioisotope, such as entire plants, animals or chromatograms are used. Usually, the dried, flat sample is placed in close contact with an X-ray film in a dark room. After allowing sufficient time for exposure, the film is developed and fixed. The image produced by the nuclear radiation can be seen on the developed film with the naked eye in the same way as an image is observed on a ordinary negative photographic film. The image produced and its relative darkness will illustrate the area and the extent of accumulation of the radiotracer in the given sample. For more detailed information about both macro and micro autoradiography, see L'Annunziata (1987).

**Materials and equipment**

1. Plant material: six week old soybean plants in pots.
2. $^{14}$CO$_2$ feeding apparatus (as in exercise 1).
3. Whole plant feeding chamber.
4. Plant pressing equipment.
5. Oven.
7. X-ray film developer.
8. X-ray film fixer.

Experimental procedure

1. Feed $^{14}$CO$_2$ to a 10-day old soybean plant, transfer into ordinary air and leave for 4-5 hours to allow distribution of photosynthates.
2. Arrange the plant in between two sheets of paper (absorbant type) in such a way that it will remain displayed well on drying. If necessary place a flat metal plate on top to ensure good pressing.
3. Place in an oven and dry at 70°C for about 24 hours.
4. Remove the dried plant, place an X-ray film in close contact with the specimen and leave in a dark room for exposure.
5. After a given period of time (about 3-5 days depending on the amount of radioactivity in the plant material), remove the X-ray film and develop in the usual manner.
6. Determine the extent to which $^{14}$C is translocated into the root system by visually observing the image produced on the X-ray film.

INFRA-RED GAS ANALYSIS TECHNIQUE

Introduction

Infra-red gas analysis (IRGA) of CO$_2$ is now a commonly used method for determining photosynthetic and respiratory CO$_2$ gas exchanges in plants. The method does not involve the use of radioisotopes. It is relatively simple and accurate, provided a suitable infra-red gas analyser is available. At present portable, battery-operated models are available which can be conveniently used in the field for field photosynthetic measurements. However, unlike with $^{14}$C, the method does not permit any biochemical or carbon partitioning studies.

Infra-red gas analysis makes use of the ability of heteroatomic gas molecules (ex. CO$_2$, H$_2$O, NH$_3$ etc.) to absorb infra-red radiation at specific wavelengths of the electromagnetic spectrum, each gas thus having a
characteristic absorption spectrum. Other gas molecules such as \( \text{O}_2 \) and \( \text{N}_2 \) which consist of two identical atoms do not interfere with measurements as they do not absorb infra-red radiation. The major absorption peak for \( \text{CO}_2 \) is at 4.25 \( \mu \text{m} \) wavelength. There are however secondary peaks at 2.66, 2.77 and 14.99 \( \mu \text{m} \) wavelengths. Unfortunately, water molecules also absorb infra-red radiation at 2.77 \( \mu \text{m} \) and since \( \text{H}_2\text{O} \) vapour is normally present in air in large quantities, this presents a problem by overlapping with the 2.77 peak of \( \text{CO}_2 \). However, this can be easily overcome by drying the air before use or by filtering out the wavelengths at which the absorption bands of the two gases overlap (Long, 1982).

An IRGA consists of three main components, the infra-red (IR) radiation source, gas analysis cells, and the detector. The IR radiation source is generally a tungsten or nichrome alloy spiral which is heated to about 600-800°C. IRGAs vary in construction but most laboratory analysers are designed to produce dual beams from a single source, allowing passage of equal amounts of IR radiation through two parallel cells. These cells are termed reference and analysis cells. The reference cell allows the passage of reference air (usually \( \text{CO}_2 \)-free air) while the sample gas passes through the analysis cell. To maximize flow of air, the inner surfaces of the cells are usually gold-plated. \( \text{CO}_2 \) present in the sample air stream will absorb a certain amount of IR radiation passing through the analysis cell thus decreasing the amount of IR radiation reaching the detectors, in comparison to the reference cell which will not absorb any IR radiation. The amounts absorbed by the detector would be inversely proportional to the amounts absorbed by \( \text{CO}_2 \) molecules in the cells. The detector signal is then rectified and amplified for display.

Recent developments in IRGA instruments have resulted in decreasing the size of analysers with single cells instead of two. The portable ADC-LCA (Analytical Development Company, England) which is an example of this type has a single sample cell with a single solid-state radiation detector. The IR radiation produced by the source passes through the cell into the detector via a thin bandpass film-filter which isolates the 4.25 \( \mu \text{m} \) absorption band. The instrument pumps sample gas and reference air alternately through the cell for periods of 2 seconds each. The radiation received in the 2 second cycle with sample air is stored and compared with that received during the next 2 seconds with reference air. The process is repeated in cycles and the difference in energy reaching the detector between half cycles is proportional to the \( \text{CO}_2 \) in the sample gas, which is rectified and amplified for display. This novel
technique of gas alternation in a single cell has resulted in the design and production of relatively small and portable instruments like the ADC LCA which is ideally suited for field measurements.

Exercise No. 3

Objective

To measure the photosynthetic and photorespiratory rates of field grown soybean and maize using the IRGA technique.

Materials and equipment

1. IRGA (ADC-LCA).
2. ADC-Parkinson leaf chamber.
3. Air supply unit.
5. ADC data lodger.
6. Plant material: field grown maize and soybean.

Experimental procedure

1. Connect the IRGA, leaf chamber and the air supply unit with suitable tubing (polythene, polypropylene or butyl tubing) in order to form an open gas-flow system (Fig. 6).
2. Check the silica gel in the air-supply unit and the soda-lime in the IRGA.

3. Switch on the air supply unit and the IRGA and set the flow rate in air supply unit to about 500 ml.min\(^{-1}\), and the flow rate in the IRGA to about 180 ml.min\(^{-1}\).

4. Activate the leaf chamber with the switch marked "AVX".

5. Set the mode selector to read zero, remove the leaf chamber cover and close the chamber without enclosing a leaf.

6. Check the zero again, and set the mode selector to "REF". The display will now show the CO\(_2\) concentration to be used in the experiment.

7. Now insert a leaf in the leaf chamber and close it.

8. Measure the CO\(_2\) concentration in the differential mode i.e. the mode selector set to "DIFF" position.

9. When a steady reading is observed (normally in 30 - 60 seconds) record the CO\(_2\) concentration.

10. Set to a) %RH, B) °C and c) PAR, to measure the % relative humidity, temperature and the photosynthetically active radiation, respectively.

11. When steady state photosynthesis is attained darken the leaf by replacing the leaf chamber cover and record the readings for about 2 minutes. The increase in CO\(_2\) concentration in the air stream will be due to photorespiratory CO\(_2\) outburst.

12. Calculate the photosynthetic and photorespiratory rates and discuss your results.

An example

In conducting a field experiment designed to measure the photosynthetic and photorespiratory rates of maize, cassava and soybean the following results (Table 9) were recorded by the investigator.

<table>
<thead>
<tr>
<th>Table 9. Experimental results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant species</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Light</td>
</tr>
<tr>
<td>Maize</td>
</tr>
<tr>
<td>Cassava</td>
</tr>
<tr>
<td>Soybean</td>
</tr>
<tr>
<td>Dark</td>
</tr>
<tr>
<td>Maize</td>
</tr>
<tr>
<td>Cassava</td>
</tr>
<tr>
<td>Soybean</td>
</tr>
</tbody>
</table>
From the data given above calculate the photosynthetic and photorespiratory rates (mg CO$_2$.dm$^{-2}$.h$^{-1}$) of the three plant species.

First, proceed to calculate the mole flow of air using the following equation:

$$F = \frac{1}{22.4} \times \frac{1}{1000} \times \frac{273}{273+T} \times \frac{P}{1.013} \times \frac{V}{1}$$  \hspace{1cm} (Equation 4)

Where,

- $F$ = Mole flow of air (moles.min.$^{-1}$)
- $V$ = Air flow rate (ml.min.$^{-1}$)
- $P$ = Atm. pressure during measurement (bars)
- $T$ = Temp. recorded during measurement (°C)

Now, substitute $F$ in the following equation:

$$NPR = \Delta C \times \frac{F}{a} \times \frac{100}{1} \times 60 \times \frac{44}{1000}$$  \hspace{1cm} (Equation 5)

Where,

- $NPR$ = Net photosynthetic rate (mg CO$_2$.dm$^{-2}$.h$^{-1}$)
- $a$ = Leaf area (cm$^2$)
- $\Delta C$ = CO$_2$ differential (vpm)

The photosynthetic and photorespiratory rates calculated using equations 4 and 5 using the data given in Table 9 are presented in Table 10.

### Table 10. Calculated photosynthetic and photorespiratory rates

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Photosynthetic rate (mg CO$_2$.dm$^{-2}$.h$^{-1}$)</th>
<th>Photorespiratory rate (mg CO$_2$.dm$^{-2}$.h$^{-1}$)</th>
<th>PR as a % of PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>53.85</td>
<td>00.63</td>
<td>1.17</td>
</tr>
<tr>
<td>Cassava</td>
<td>24.08</td>
<td>07.60</td>
<td>31.56</td>
</tr>
<tr>
<td>Soybean</td>
<td>21.97</td>
<td>08.45</td>
<td>38.46</td>
</tr>
</tbody>
</table>
REFERENCES


USE OF NEUTRON WATER AND GAMMA DENSITY GAUGES IN SOIL WATER STUDIES

C. KIRDA
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna

INTRODUCTION

Water is one of the most important input required in agricultural production. The water requirement for plant growth is met from soil water stored in the plant root zone. In high rainfall areas and temperate regions, soil water is continuously replenished with rainfall and is therefore not a major constraint in agricultural production. However, in arid and semiarid regions, or areas of low and erratic rainfall which comprise one third of the global land area, water scarcity is the major constraint to increased crop yields. Therefore under the conditions of highly variable climatological environment and chronically deficient rainfall of the arid zones, sustainable food security can not be obtained if the agricultural practices do not address effective use of the most precious and yet uncertain resource, water.

Irrigation is one of the means available for maintaining optimum levels of soil water in the plant root zone. Although only 20% of all cultivated land in the world is under irrigation, it provides nearly 40% of all crop output. Therefore investments for irrigation receive top priority. For example, in the Soviet Union about 1 million hectares of newly irrigated land area have been developed each year. In Hungary, irrigated areas increased ten fold since World War II. In the US they have more than doubled in the last 10 years. However it has become a matter of serious concern in recent years that, despite their high cost (US$2000 - 10000/ha), the performance of many irrigation projects have not been fully attained. As a result of inadequate water management both at farm and system level, much less area was often irrigated than actually planned, and therefore crop production increase was below the project targets. Low irrigation efficiencies and excess water application in the fields compounded with seepage of water along the irrigation networks caused rising of ground water table, which in turn, triggered soil salinity problem in irrigated areas. Conversely, in many parts of the world, irrigation is not available either because of scarcity of irrigation water resources or economic constraints hindering expansion of irrigated agriculture. Thus, agricultural scientists, particularly in arid and semiarid
regions, need alternative avenues for maximizing the use of the soil water available for plant growth. The measurement and management of soil water are therefore of great importance in efforts to test new innovations that could be incorporated into the existing farming systems to meet the water requirement and demands of food for ever increasing human population.

Irrigation practices should be improved to increase effective use of water and thereby increasing irrigated areas as well as securing soil productivity under irrigated agriculture. Under dry farming systems of rainfed agriculture, different tillage practices should be tested for improved soil water conservation and rain harvesting. The research work addressing the above mentioned problems requires methods to measure soil water content accurately and conveniently. In the following sections, the methods which are currently used to measure field soil water content were discussed.

METHODS FOR MEASURING SOIL WATER CONTENT

Direct Method

This is based on gravimetric sampling and requires sample containers, a balance and an oven. It is a simple approach which can give precise measurements. However, its use is laborious and results are only available on the following day. Additionally, number of samples and their associated locations influence the accuracy of field mean values.

Indirect Methods

1. Tensiometers

Water moves from areas of high energy to areas of low energy. Energy status of soil water can be measured with tensiometers in units of energy per unit of water. It is simply expressed as mechanical work equivalent of energy required to change the status of water from A to B as shown in Fig. 1.
1. Flow of water from A to B

\[ A \rightarrow B: + \Delta \varepsilon \]

Energy is produced

DAM

2. Pumping ground water

\[ A \leftrightarrow B: - \Delta \varepsilon \]

Energy is needed

Pump

3. Transpiration

\[ A \leftrightarrow B: - \Delta \varepsilon \]

Energy is needed

FIG. 1.

Energy of water can be expressed in different units which are shown in Table 1.

Table 1. Units to measure energy of water

A. Basic units

<table>
<thead>
<tr>
<th>Unit of measurement</th>
<th>Unit of water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Energy, erg/g</td>
<td>Mass, g</td>
</tr>
<tr>
<td>2. Pressure, dyne/cm²</td>
<td>Volume, cm³</td>
</tr>
<tr>
<td>3. Length, cm</td>
<td>Weight, dyne</td>
</tr>
</tbody>
</table>
Table 1. (cont.)

B. Conversion formulas

<table>
<thead>
<tr>
<th>Energy of water</th>
<th>Potential energy</th>
<th>Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>erg/g</td>
<td>joule/g</td>
<td>Bar</td>
</tr>
<tr>
<td>-980h</td>
<td>-9.80E-09h</td>
<td>h/1022</td>
</tr>
<tr>
<td>h/1035</td>
<td>--</td>
<td>0.9869P</td>
</tr>
<tr>
<td>P(in bars)</td>
<td>1.0E-06P</td>
<td>1.0P</td>
</tr>
<tr>
<td>--</td>
<td>0.1P</td>
<td>100P</td>
</tr>
</tbody>
</table>

In soil water studies, energy of water is usually measured as the equivalent of water energy per unit weight and it is called hydraulic head (cm of water):

\[ H = h + z \]

where \( H \) is the total hydraulic head (cm), \( h \) the pressure head and \( z \) is the gravitational head. Under water-logged or saturated conditions, the pressure head \( h \) is positive and is measured with piozometers; under unsaturated conditions, however, it is negative and is measured with tensiometers. The negative pressure head takes different names in published literature, and among these are capillary head, matrix head or tension.

The tensiometer is a very simple equipment. It has the following three major parts (Fig. 2): (1) a porous cup, (2) a tube and (3) a mercury manometer (or a vacuum gauge).

Soils can hold varying amounts of water at a given capillary head, depending on their physical and chemical characteristics. A graph describing interdependence of soil water content and capillary head is called "soil water characteristics curve" (Fig. 3). Soil capillary pressure measured with tensiometers can be easily converted to soil water content with soil water characteristics curves.

2. Resistance and Capacitance Blocks

They are based on resistance or capacitance measurements made between two metal conductors imbeded into a porous material either made of nylon or gypsum (Fig. 4). The blocks are placed at different soil depths and measuring sites of interest. An equilibrium exists between water within the porous blocks and water in soil. If soil in the vicinity of the blocks is dry, waterescapes.
FIG. 2. Major parts of tensiometers.

FIG. 3. Soil water characteristics curve.

FIG. 4. Major parts of resistance blocks.
from the blocks and a new equilibrium is established. Soil water content can be measured indirectly with these porous blocks using calibration curves that relates water content to either resistance or capacitance measured in the blocks (Fig. 5).

3. Time-domain Reflectometry

The newly developed time-domain reflectometry (TDR) is essentially based on measurement of propagation velocity of an electromagnetic pulse (voltage step) imposed between two transmission lines (wave guides) inserted into the soil where measurement is needed. The electromagnetic pulse traveling along the transmission lines reflects back to its source when it reaches to the end of the lines. Therefore the path traveled is twice the length L (m) of the lines. The propagation velocity of the pulse is

\[ v = \frac{2L}{t} \]

where \( t \) (sec) is the total travel time of the electromagnetic pulse and it is measured directly by the TDR unit. The propagation velocity \( v \) is related to the bulk dielectric constant of the medium:

\[ v = \frac{c}{(K_d)^{1/2}} \]

where \( K_d \) is the dielectric constant, and \( c \) the velocity of light in free space \((3 \times 10^8 \text{ m/sec})\). The TDR units which are now commercially available measure the bulk soil dielectric constant using the relation:

\[ K_d = \left( \frac{ct}{2L} \right)^2 \]
It has been shown that the soil dielectric constant is primarily a function of soil water content, and weakly depends on soil type, soil density, soil temperature and salt content (Top and Davis, 1985).

Measurements of soil water content with this method gives high correlation when compared to gravimetric sampling (Top et al., 1984). It has been shown that the TDR could also be used to measure bulk soil electrical conductivity (i.e., soil salinity) (Dasberg and Dalton, 1985; Dalton et al., 1984).

4. Nuclear Methods

There are essentially two nuclear methods used in soil water studies: (1) neutron scattering (or thermalization), and (2) gamma ray attenuation and back scattering. The following sections are devoted to the nuclear methods.

NEUTRON SCATTERING METHOD

Neutrons are uncharged particles (i.e., without positive or negative charges) of mass 1.008982, slightly higher than that of protons. They decay into a proton and an electron with a half life of 12.8 minutes. Their classification is based on their kinetic energy levels (Table 2).

<table>
<thead>
<tr>
<th>Description</th>
<th>Kinetic energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast neutrons of high energy</td>
<td>&gt; 10 MeV</td>
</tr>
<tr>
<td>Fast neutrons</td>
<td>10 KeV - 10 MeV</td>
</tr>
<tr>
<td>Intermediate</td>
<td>100 eV - 10 KeV</td>
</tr>
<tr>
<td>Slow neutrons</td>
<td>0.03 eV - 100 eV</td>
</tr>
<tr>
<td>Epithermal neutrons</td>
<td>1 eV</td>
</tr>
<tr>
<td>Thermal neutrons</td>
<td>0.025 eV</td>
</tr>
</tbody>
</table>
Neutron interactions with matter are rather complex. Calculation of neutron fluxes involves probability and diffusion theories. However, for simple practical engineering applications, these interactions can be grouped as:

i. inelastic scattering  
ii. elastic scattering  
iii. absorption

Inelastic scattering involves transformation of kinetic energy into some other form of energy and it is important for fast neutrons. Interactions which interest us are elastic scattering and "absorption" (capture). With "absorption", neutrons end their existence as a result of a nuclear reaction, and this is often used as a basis for detection of neutrons in designing of neutron detectors. Scattering occurs as the principal interactions of fast neutrons; whereas, absorption occurs as interactions of slow neutrons. The scattering of fast neutrons produces slow neutrons. The slow neutrons are therefore the end result of elastic scattering of fast neutrons through interactions with nuclei of surrounding atoms present in the media. Probability of various means of interactions of neutrons with other elements is measured with what is called "cross sections". Table 3 gives neutron cross section of various soil elements. Probability of interactions of fast neutrons with common soil elements is approximately the same (Table 3). However, when low kinetic energies are reached through successive elastic scatterings, cross section of hydrogen increases about 40 fold. Similarly cross sections for oxygen, chlorine and iron also increase. Gardner and Kirkham (1952) reported that the weight of an atomic nucleus also influences the scattering process as much as cross sectional areas. The energy loss through elastic scattering is greater, the lighter the nucleus of the interacting element. It can therefore be concluded that hydrogen is the primary element causing scattering and slowing down of fast neutrons in moist soils. Multiple scattering due to elastic collisions of neutrons with nuclei of elements reduce neutron energies to the point where they are in thermal equilibrium with a gas at 20°C. Neutrons which have reached this energy level may gain as much energy as they lose through further collisions. They are considered slow neutrons of thermal energy state. Neutrons require the least number of collisions with the element H to slow down to thermal energy state (Table 3). This characteristic is used as a base to measure soil water content with neutron scattering technique using the assumption that all the H atoms stem from water molecules. In soils high in chloride and iron, this
Table 3. Neutron cross sections of important soil elements in barns per atom (10^{-24} \text{ cm}^2 \text{ per atom})

<table>
<thead>
<tr>
<th>Element</th>
<th>Scattering fast neut. 2.5 MeV</th>
<th>Scattering slow neut. 0.025 eV</th>
<th>Absorption slow neut. 0.025 eV</th>
<th>Collisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>2.5</td>
<td>81.5</td>
<td>0.33</td>
<td>19.0</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>7.5</td>
<td>755.00</td>
<td>109.2</td>
</tr>
<tr>
<td>C</td>
<td>1.6</td>
<td>5.5</td>
<td>3.20</td>
<td>120.6</td>
</tr>
<tr>
<td>N</td>
<td>1.0</td>
<td>11.4</td>
<td>1.90</td>
<td>139.5</td>
</tr>
<tr>
<td>O</td>
<td>1.5</td>
<td>4.2</td>
<td>0.00</td>
<td>158.5</td>
</tr>
<tr>
<td>Na</td>
<td>2.6</td>
<td>3.4</td>
<td>0.50</td>
<td>224.9</td>
</tr>
<tr>
<td>Mg</td>
<td>2.0</td>
<td>3.7</td>
<td>63.00</td>
<td>237.4</td>
</tr>
<tr>
<td>Al</td>
<td>2.5</td>
<td>1.5</td>
<td>0.23</td>
<td>262.8</td>
</tr>
<tr>
<td>Si</td>
<td>3.2</td>
<td>2.4</td>
<td>0.13</td>
<td>273.3</td>
</tr>
<tr>
<td>Cl</td>
<td>2.7</td>
<td>16.0</td>
<td>31.60</td>
<td>343.3</td>
</tr>
<tr>
<td>K</td>
<td>3.8</td>
<td>2.2</td>
<td>1.97</td>
<td>378.0</td>
</tr>
<tr>
<td>Ca</td>
<td>4.9</td>
<td>3.2</td>
<td>0.43</td>
<td>387.3</td>
</tr>
<tr>
<td>Fe</td>
<td>3.0</td>
<td>11.8</td>
<td>2.53</td>
<td>537.2</td>
</tr>
</tbody>
</table>

* Adapted from van Bavel (1963)

assumption may be significantly influenced by the secondary effects of chloride and iron which also slow down neutrons when they occur in high quantities.

Neutron Water Gauges

In neutron water gauges, neutrons are usually produced through (\alpha,n), (\beta,n) or (\gamma,n) reactions. For example, in some neutron gauges, neutrons are produced as a result of the following nuclear reaction:

\[ ^{9}\text{Be}(\alpha,n)^{12}\text{C} \]

Neutrons produced by this reaction have a spectrum of energies from 0 to 10 MeV with an average value of 4.5 MeV. The \(^{12}\text{C}\) is left in excited state which in turn produces a photon of 1 - 10 MeV and goes eventually down to ground state. The neutron moisture gauge essentially measures the number of slowed down neutrons (n).
Because of the following relations:

\[
\begin{align*}
\text{cpm} &= f(n) \\
n &= g(H) \\
H &= s(\theta),
\end{align*}
\]

where \( f, g \) and \( s \) designate different functions with the indicated arguments, one can demonstrate that generally a linear relation exists between neutron count rate (cpm) and volumetric soil water content (\( \theta \)). If such a calibration relation is constructed which is valid for a particular condition, soil water status of plant root zone can be continuously, nondestructively and easily monitored.

Calibration

Neutron gauges only measure slow neutrons. Because it is not easy to describe the interactions between slow neutrons and different soils, a calibration curve is needed to convert the neutron counts, measured with neutron gauge, to water content. The following equation

\[
\theta = a + bR
\]

is commonly fitted to the calibration data of the neutron gauges, where \( \theta \) (cm\(^3\)/cm\(^3\)) is the estimated volume fraction of free soil water (water released on drying at 105°C), \( R \) is the ratio of neutron count rate \( C \) in the soil to the count rate \( C_s \) in a standard medium, usually water, and \( a \) and \( b \) are constants. However, calibration curves developed are only valid for a given soil type for which they are derived. Soil chemical composition and soil density have significant influence on calibration curves of neutron gauges. Therefore users of these type of gauges must be aware of various sources of errors involved in their calibration. Greacen et al., 1981 reviewed the methods used in calibration of neutron gauges and discussed various sources of errors encountered in the calibration.

1. Field Calibration

Field calibration is carried out by installing access tubes in natural field soils. First neutron count rates are measured at different soil depths known to vary in chemical composition and bulk density. Volumetric soil samples are then taken from the same soil depths where neutron count rates were measured. Volumetric soil samples should be taken as close as possible to
the neutron gauge access tube so that the sample represents a volume of soil which comes from the sphere of influence of the slow neutrons (Fig. 6). It is recommended that a couple of samples circling the access tube from each soil depth is taken. Mean volumetric soil water content of the samples, measured gravimetrically is later regressed with neutron count rate at that specific soil depth. In practice however, neutron count rate ratios, not the count rates, are used. For this propose, neutron count rate is first measured in a standard medium, which is usually water, before field measurements are started. Neutron count rate ratios (R) are calculated by dividing neutron count rates obtained in the field with the neutron count rate of the standard medium.

FIG. 6. Sphere of influence of thermal neutrons in neutron gauge.

Measurement made at one site during the field calibration provides only a single point (i.e. one volumetric water content corresponding to one neutron count rate ratio). More points can be obtained at other randomly selected measuring sites of the field. Optimum number of points for the calibration procedure varies depending on field variability. The calibration curve should cover a wide range of changes in soil water content, which could occur under normal agricultural practices.

In some cases, users prefer to calibrate their equipment as a supplementary work to their field experiments. They initiate their field research work prior to calibration of the equipment. They install the access tubes right in the experimental plots where the crops are to be grown. Soil
core samples are taken outside of the experimental plots for the calibration of the equipment. Neutron count rates taken right in the experimental plots are then regressed against soil water content measurements made with the core samples which may sometime be taken 5 to 10 m away from the access tubes to prevent damaging of the experimental plots. Alternatively, soil water content may be measured using disturbed soil samples where one can measure soil water content on weight basis which is later corrected to volume fraction by multiplying with soil bulk density, measured in the experimental plots at the end of the experiment. This method of calibration provides more data points than when soil samples are taken directly next to the access tubes. However here, soil density and chemical composition of the sites where gravimetric soil samples are taken may be significantly different from the sites where the access tubes are, i.e., in the center of the experimental plots. Future research work should evaluate if calibration of the gauges using soil samples taken at larger distances from the access tubes gives significantly different results from the calibration when the soil samples are taken next to the access tubes. Many early publications used this later method of calibration; however, to our knowledge, no one has yet compared the two different approaches, taking soil samples very close to the access tubes or at some distance from the tubes.

Correlation coefficients of the calibration curves, may sometime be very low, particularly in gravelly soils. This is attributed to field heterogeneity and inherent problems associated in gravimetric water content measurements in gravelly soils (Babalola, 1978). Users should not be discouraged with low correlation coefficients as long as the error of the estimate is within the range of 10 to 15% of the field mean water content.

Field calibration relations carries combined errors arising from field soil heterogeneity and compaction of volumetric soil samples. The following sources of errors are most important in field calibration procedure:

1. Soil water content measured by direct soil sampling does not necessarily represent soil water content within the sphere of influence of a neutron gauge,

2. Volumetric soil samples needed for calibration may be compacted at some level but there are no easy means of measuring or estimating the resulted error,
The field calibration relation is influenced by soil horizons which differ in chemical composition and soil bulk density (Lal, 1974; Marais and Smith, 1960).

2. Laboratory Calibration

Van Bavel (1963) suggested the use of homogeneous volumes of soil under laboratory conditions to calibrate neutron gauges. However, it was shown that soil containers have to be sufficiently large for the neutron count rates to be independent of the soil volume (Van Bavel et al., 1961). It was suggested that 1 m$^3$ of soil seems to be the minimum volume for low soil water content. Size constraint may not be very critical for water contents over 20 \% volume fraction (Van Bavel, 1963). The following steps should be taken in the laboratory calibration procedure:

1. The sample should be prepared as homogenous as possible with regard to both soil water content and bulk density,

2. Soil water content measurement should be based on whole volume of the soil sample, or large sub-samples must be used,

3. Separate samples should be prepared for each point of measurement for the calibration relation.

3. Calibration by Simulation

In laboratory calibrations, large soil samples are required and the work involved is too laborous. As a result relatively more permanent and easily constructed standards made either from neutron moderators or combination of moderators and absorbers are suggested. The following mixtures are some examples used for this propose:

1. Mixture of alum ($\text{Al}_2\text{Ti}((\text{SO}_4)_2\cdot12\text{H}_2\text{O})$) and sand in large barrels to correspond to certain known soil water content (McGuinness et al., 1961),

2. Paraffin and sand mixtures (Hauser, 1962),

3. Liquid standards containing either boric acid or cadmium chloride (Van Bavel et al., 1961).
However one has to note that calibration with simulation standards can only be used as a supplement to known calibration relation in soils. Thus they can not substitute the actual calibration relation and only help to evaluate the behavior of the equipment in the long run. Calibration with simulation standards also provides valuable data for interchange of calibration curves developed for a given type of probe. This is very useful when a user buys a new gauge and he wants to adapt the calibration curve of his old gauge for the new one. This is only possible if the user had calibrated his old equipment both in the field and using simulation standards. One can estimate coefficients of the calibration curve for the new probe with measurements made only in the simulation standards. Nakayama and Reginato (1982) used plastic blocks as intermediate standards for transferring the field calibration from one probe to an other. However, the procedure is applicable only for those gauges of similar geometry, detector type and source strength (Reginato and Nakayama, 1987).

4. Theoretical Calibration

This method is based on a numerical model describing interactions of thermal neutrons with soil matrix (Couchat et al., 1975). The method requires measurement of neutron capture characteristics of soils. However, the neutron capture characteristics of field soils can only be measured in specific laboratories. The final stage of calibration calculations, has once again to rely on a reference calibration curve, prepared for a specific probe. Vachaud et al. (1977) showed that the theoretical calibration was well suited for determining the calibration curve of clay soils and of heterogeneous gravelly soils for which field calibration may present difficulties. The method seems to be promising; however, further effort should be continued to improve and simplify measurement of neutron capture characteristics of field soils.

Exercise 1

Field calibration of a neutron gauge was carried out in research fields of the Agency's Laboratory, Seibersdorf, as a training exercise. Forty measuring sites were selected in the field. First, measurements were made in half of the sites which were randomly selected, when soil was relatively dry. Measurements were completed in the remaining sites, after the field was irrigated to increase field soil water content. The calibration data is given in Table 4. Calculate for all sites neutron count ratio \( R \) by

\[
R = \frac{C}{C_s}
\]
Table 4. Field calibration data of CPN 503DR gauge.

<table>
<thead>
<tr>
<th>Site No</th>
<th>Count C</th>
<th>Water Cont. θ (%)</th>
<th>Site No</th>
<th>Count C</th>
<th>Water Cont. θ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Std. Count \( (C_o) \): 12257

2       | 13422   | 33.76             | 1       | 19581   | 49.25             |
3       | 13555   | 34.09             | 5       | 15771   | 39.66             |
4       | 11830   | 29.75             | 6       | 14805   | 37.23             |
7       | 12980   | 32.64             | 8       | 15639   | 39.33             |
9       | 18311   | 46.85             | 10      | 19293   | 48.52             |
12      | 17073   | 42.94             | 11      | 19569   | 49.22             |
14      | 13951   | 35.09             | 13      | 17108   | 43.03             |
15      | 12982   | 32.65             | 16      | 16328   | 41.06             |
18      | 15668   | 39.38             | 17      | 18184   | 45.73             |
19      | 15293   | 38.46             | 20      | 19696   | 49.52             |
22      | 12910   | 32.47             | 21      | 15611   | 39.26             |
23      | 11136   | 28.56             | 25      | 12954   | 32.56             |
24      | 12847   | 32.31             | 26      | 14340   | 36.06             |
27      | 12606   | 31.78             | 28      | 14024   | 35.27             |
29      | 12354   | 31.07             | 30      | 14893   | 37.46             |
32      | 11873   | 29.86             | 31      | 14235   | 35.80             |
34      | 12034   | 30.27             | 33      | 15083   | 37.73             |
35      | 12107   | 30.45             | 36      | 14535   | 36.56             |
38      | 13108   | 32.95             | 37      | 14413   | 36.25             |
39      | 13307   | 33.47             | 40      | 14604   | 36.73             |

Using \( R \) as an independent variable, calculate the regression equation which estimates soil water content \( \theta \) with neutron count ratios.

ISSUES DEALING WITH THE USE OF NUCLEAR GAUGES

1. **Calibration in Gravelly Soils**

Soil water content measured with neutron gauges is an average value integrated over a spherical volume of the soil which is called "zone of influence" of the gauge. The measurement gives volume fraction of water
existing in the soil. If the soil is very gravelly water content measurements made with neutron gauges are not necessarily what plant roots are exposed to, because the gauge simply gives an average water content integrated over a large volume of soil, including stones. Water uptake by roots comes from the soil fraction. Therefore one has to correct calibration curves if water, as fraction of fine soil particles, is to be measured. The reports by Lal (1977) and Babalola (1976) should be consulted for further information on the influence of soil gravel content on neutron calibration curves.

2. Influence of soil cracking on neutron gauge measurements

Some soils crack due to shrinkage upon drying. In such instances, the neutron access tubes are exposed to air in the measuring sites, and the problem is further complicated by increase of soil bulk density as the soil dries. Therefore, the users of the gauges should be concerned about erroneous measurements obtained under such conditions. However, Hodgson and Chan (1981) reported that corrections of the calibration curves to account for soil cracking and density changes do not improve the calibration very significantly. Therefore, it is not necessary to worry about this issue as long as the calibration curve covers the complete range of soil water content where cracking and shrinkage of the soil are observed.

3. Number of Measuring Sites for Neutron Gauges

If one has prior knowledge of site variability as standard deviation of estimated water storage, it is very simple to calculate the number of sites needed to achieve a given level of accuracy in the measurement, by using the procedure described by Johns et al. (1981). If the measurements are assumed to follow a normal probability distribution, then one can write

\[ N = \frac{t^2 S^2}{D^2} \]  

[1]

where \( N \) is the number of measuring sites, \( t \) the tabulated value of \( t \) for the probability level \( p \), \( S^2 \) the variance of the measurements and \( D \) is the specified deviation from the true mean which is to be estimated. As an example, for 95% probability, using an approximate \( t \) value of 2.0, for \( S = 10 \) mm and \( D = +5 \) mm

\[ N = \frac{4 \times 100}{25} = 16 \text{ sites.} \]
In general practice, the aim of soil water studies is to compare different treatments. Therefore, the essence is to estimate how many measuring sites are needed in each treatment, to achieve a specified least significance difference (L.S.D.) between the treatments. Again following the procedure described by Johns et al. (1981).

\[
N = 2t_p^2 \frac{S^2}{(L.S.D.)^2}
\]  \hspace{1cm} [2]

where (L.S.D.) is the 5 \% least significant difference. As an example, for \( S = 10 \) mm, to achieve a 5 \% L.S.D. of 10 mm, one would need

\[
N = 2 \times 4 \times 100/100 = 8
\] measuring sites.

ACCESS TUBE INSTALLATION

Aluminum, aluminum alloy, brass and stainless steel tubes are among the most commonly used material for neutron access tubes. All aspects of access tubes and their installation were discussed by Eales (1969). This section is prepared based on his work.

The following factors affect the choice of the tubing material:

(1) Soil chemistry,
(2) Durability of tubing material,
(3) Depth of access tube installation.

Aluminum is the most transparent material to thermal neutrons. Brass, although may reduce the count rate slightly, but it is less corrodible in sodium affected soils. A stainless steel tube is the most durable material, but neutron count rates are reduced significantly due to the large absorption cross-section.

Bottom end of the access tubes should ideally be closed by a tapered plug of the same material. When they are installed, the tops of the tubes must also be closed with appropriate caps.

For installation of access tubes, hand operated soil augers should be used. Motor powered mechanical augers may cause drastic disturbance of the soil structure owing to churning with caught-up stones, compression,
over-sizing etc. The following equipments are needed for an ideal installation of access tubes:

(1) Aluminum plate,
(2) Guiding-tube made of steel with cutting edge,
(3) Hand auger,
(4) Tube extractor,
(5) Access tubes.

The following steps are taken for the access tube installation:

(1) Place the aluminum plate on the site selected for installation of the access tube,
(2) Working through the hole at the center of the aluminum plate, drill 12 to 15 cm with a hand auger,
(3) After the auger is withdrawn, insert the steel guiding tube to the same depth as the auger reached. Make sure that the tube is placed vertically,
(4) With a hand auger, once again drill further 12 to 15 cm depth, withdraw the auger and hammer down the guiding tube to the bottom of the hole,
(5) The loose soil can be taken out with an auger through the guide tube,
(6) Continue this procedure until the required depth is reached,
(7) After insertion of the guiding tube to the required depth, it is withdrawn with care to prevent a widening of the auger hole,
(8) Insert the permanent access tube by ramming gently into the soil,
(9) Cut the top of the access tube at the desired height.

The guide tube can sometimes be easily removed by twisting and pulling with a tommy bar. But in some cases it must be hauled out with a specially designed grip and puller jack. It is essential to rotate the tube particularly while withdrawing it from wet and heavy clayey soils, so as to avoid enlarging the access tube hole.

Prebble et al. (1981) summarized different procedures used in access tube installation, which would normally vary depending on the nature of the work and soil types.
Gamma radiation is electromagnetic radiation and as a result, has more penetrating effect than other types of radiation with similar energy. Absorption of gamma rays in matter depends mainly on (1) photon energy, (2) proton number and (3) density of absorbing material. Various types of absorption are described below:

1. **Photoelectric absorption**

   This is mainly for gamma radiation of relatively low energy and absorbing materials of high atomic number (i.e., high number of protons in the nucleus). Gamma rays (photons) interact with those electrons which are closest to the nucleus of the absorbing atom, K- or L- shell electrons. When gamma ray is completely absorbed, an electron with energy slightly less than that of the absorbed gamma ray is ejected (Fig. 7a). The energy difference is equal to the binding energy of the electron.

![Photoelectric absorption](image)

**FIG. 7.** Different modes of interactions with gamma rays and matter.

2. **Compton effect**

   Compton effect is an interaction of gamma photon with an outer electron of the absorber atom. Part of the energy is absorbed (transferred to the electron) and the photon is scattered off in a new direction. After multiple scatterings, the photon goes through photo electric absorption (Fig. 7b).

3. **Pair-production**

   This can only occur when gamma photons have at least initial energies of 1.02 MeV. Here gamma photons interact with the positive field of the nucleus of the absorbing atom. Its energy is completely used up by producing a
positron and an electron pair. Threshold energy for pair production is 1.02 MeV. Both positron and electron cause ionization along their respective paths (Fig. 7c).

**GAMMA RADIATION PROBE**

Based on known interactions of gamma rays with orbital electrons of soil constituents, one can measure soil density. Gamma photons with energy less than 1 meV interact with surrounding electrons with so-called "compton effect" and/or photoelectric absorption. As the number of electrons increase per unit volume of soil, the compton scattering power of the medium increases proportionally with increase of soil density. Thus as the electron density increases in the medium, the probability of multiple scattering will increase. This condition implies that the probability of photoelectric absorption will also increase, thus causing a reduction of gamma photons reaching the detector. Commercially available gamma probes work mainly on the two principles of gamma-ray attenuation or gamma back scattering.

1. **Gamma-ray Attenuation Technique**

When monoenergetic and collimated gamma rays pass through a given material, the intensity of incident beam (I) is attenuated owing to the interaction of gamma photons with the material. The attenuated fraction of gamma rays (dI/I) is directly proportional to the thickness of the material X, as described below:

\[ \frac{dI}{I} = -kX \]  

[3]

In this relation, \( k \) is referred to as linear attenuation coefficient which is the sum of several attenuation coefficients representing individually occurring attenuation processes (Ferraz and Mansel, 1979):

\[ k = k_c + k_e + k_p + \ldots \]  

[4]

where subscripts c, e and p represent compton, photoelectric and pair production effects. The sum of these coefficients indicates the total probability of attenuation of gamma rays of specific energy during transmission through a given material of some specific chemical composition.
Gamma ray attenuation coefficients can also be expressed as a mass attenuation coefficient, which is simply the linear coefficient \( k \) divided by the density \( \rho \) of the material absorbing the gamma rays.

Attenuation of gamma rays can be described by the following relations:

\[
\frac{dI}{I} = - \mu \rho dX \quad [5]
\]

Integration of equation [5] yields

\[
\ln \left( \frac{I}{I_0} \right) = - \mu \rho X \quad [6]
\]

\[
\frac{I}{I_0} = \exp(- \mu \rho X) \quad [7]
\]

where \( I_0 \) and \( I \) are initial and attenuated gamma ray counts, respectively.

Field soils are composed of solid particles, liquid water and air space. If one ignores attenuation by soil air, then

\[
\frac{I}{I_0} = \exp[-( \mu_w \theta + \rho_s \mu_s)X] \quad [8]
\]

In practice americium-241, cesium-137 or cobalt-60 is used as a gamma source in laboratory gamma attenuation equipment. Approximate gamma attenuation coefficients of water and soil for monoenergetic radiation from the above sources are given in Table 5.

Table 5. Approximate gamma attenuation coefficients of water and soil.

<table>
<thead>
<tr>
<th>Source</th>
<th>Energy</th>
<th>Water</th>
<th>Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Am-241</td>
<td>60 keV</td>
<td>0.200</td>
<td>0.25 - 0.42</td>
</tr>
<tr>
<td>Cs-137</td>
<td>662 keV</td>
<td>0.085</td>
<td>0.077</td>
</tr>
</tbody>
</table>

The following can be measured with laboratory gamma attenuation equipment:

1. Water content if soil bulk density and \( \mu_s \) are known.
2. Soil bulk density if water content and \( \mu_w \) are known.
(3) Change of soil water content under non-swelling conditions using the relation

\[ \Delta \theta = \frac{1}{\mu_w X} \ln \left( \frac{I/I_0}{1/(I/I_0)_2} \right) \]  \hspace{1cm} [9]

where subscripts 1 and 2 are for initial and later stage gamma attenuation measurements.

(4) Soil water content and density can be measured simultaneously if dual gamma source is used (Ferraz and Mansell, 1979).

ii. Gamma Back Scattering

Gamma rays from monoenergetic gamma sources go through successive scatterings before reaching a detector (Fig. 8). Primary interactions of gamma rays in these types of probes are compton scattering and photo electric absorption. Wet soil density can be obtained directly with this type of equipment using simple empirical calibration relation of the type shown in Figure 8.

![Figure 8](image)

**FIG. 8.** Principle of commercial gamma probes working either based on backscattering or attenuation.
Gamma probes which are presently in use are coupled with neutron probes to measure soil dry density and volumetric water content simultaneously.

**USE OF NUCLEAR GAUGES IN SOIL WATER STUDIES**

Nuclear methods namely neutron moisture and gamma density probes have rather wide area of application in agricultural research. They are nondestructive methods, and once calibrated, are very easy to use. For certain type of work, they can be used directly, without additional calibration, beyond that supplied by the manufacturer. The following section deals with their various uses in agricultural research.

**Water Storage in the Plant Root Zone**

Information on water storage in the plant root zone is widely used for decisions on the most suitable types of cropping systems in a given region. Effective rainfall at the end of a rainy season can easily be assessed by changes in water storage before and after the rains. Water retention capacity of soils influencing water availability to plants can be calculated if one knows the changes of water storage in the plant root zone before and after irrigation.

Only soil water content distribution profiles within the plant root zone are needed to calculate the amount of soil water stored ($S(t)$). This is easily assessed with neutron moisture probes using the following equation,

$$S(t) = \int_{0}^{L} \theta dz \quad [10]$$

$$S(t) = \Sigma \theta \Delta z \quad [11]$$

where the right sides of the equations are the area under the soil water profile curve (Fig. 9).

**Exercise 2**

A field experiment was irrigated with 25 mm water by sprinkler irrigation system. Calculate whether the applied water was adequate to wet 50 cm of the soil profile using soil water content data in Table 6. Calculate also the change of soil water storage, $S$ and comment on evaporative loses of water during irrigation.
FIG. 9. Soil water storage in plant root zone.

Table 6. Neutron gauge data before and after irrigation.

<table>
<thead>
<tr>
<th>Depth Z (cm)</th>
<th>Neutron count, C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>15</td>
<td>--</td>
</tr>
<tr>
<td>20</td>
<td>13727</td>
</tr>
<tr>
<td>25</td>
<td>12900</td>
</tr>
<tr>
<td>30</td>
<td>12688</td>
</tr>
<tr>
<td>35</td>
<td>11090</td>
</tr>
<tr>
<td>40</td>
<td>9615</td>
</tr>
<tr>
<td>45</td>
<td>9600</td>
</tr>
<tr>
<td>50</td>
<td>10900</td>
</tr>
<tr>
<td>55</td>
<td>10500</td>
</tr>
<tr>
<td>60</td>
<td>9900</td>
</tr>
<tr>
<td>65</td>
<td>9998</td>
</tr>
<tr>
<td>70</td>
<td>9980</td>
</tr>
<tr>
<td>75</td>
<td>9950</td>
</tr>
<tr>
<td>80</td>
<td>10000</td>
</tr>
<tr>
<td>85</td>
<td>9990</td>
</tr>
<tr>
<td>90</td>
<td>9980</td>
</tr>
<tr>
<td>95</td>
<td>9990</td>
</tr>
<tr>
<td>100</td>
<td>9900</td>
</tr>
</tbody>
</table>

Std. Count. Cs

12343

12484

Neutron gauge calibration equation:

θ = 1.4919 + 16.401R (%)
Plant Rooting Habits

Information on plant rooting habits is valuable for irrigation management practices and fertilizer application. For instance, irrigation engineers use a concept called "effective rooting depth" in irrigation system designs, which is the maximum soil depth providing 80% of water used by plants. The effective rooting depth is needed to calculate irrigation water requirement. Adequacy of irrigation water application can best be evaluated if one knows the effective plant rooting depth. Plant rooting habits can also be used in the selection of plant genotypes adapted to drought.

Depths of soil water depletion can be used as indirect means of estimating plant root activity distribution in soil profiles (Levin et al., 1973; Castle and Krezdom, 1977; Stone et al., 1976). A review by Bohm (1979) compares methods developed to study root systems.

Exercise 3

Table 7 shows soil water content distribution profiles for two different crops planted in the same field. Plant nutrition status as well as physical and chemical characteristics of the soil were identical for the two crops. Initially, both sites were adequately irrigated and soil water content was brought to field capacity. Table 8 which is complementary to Table 7 shows changes of soil water storage S in each 10 cm soil layer 2 weeks after irrigation.

Table 7. Soil water distribution profiles under two different crops.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Initial $\theta$ (cm$^3$/cm$^3$)</th>
<th>$\theta$ (cm$^3$/cm$^3$), after 2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plant 1</td>
</tr>
<tr>
<td>10</td>
<td>0.50*</td>
<td>0.40*</td>
</tr>
<tr>
<td>20</td>
<td>0.55</td>
<td>0.44</td>
</tr>
<tr>
<td>30</td>
<td>0.54</td>
<td>0.46</td>
</tr>
<tr>
<td>40</td>
<td>0.52</td>
<td>0.48</td>
</tr>
<tr>
<td>50</td>
<td>0.52</td>
<td>0.48</td>
</tr>
<tr>
<td>60</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>70</td>
<td>0.50</td>
<td>--</td>
</tr>
<tr>
<td>80</td>
<td>0.47</td>
<td>--</td>
</tr>
<tr>
<td>90</td>
<td>0.45</td>
<td>--</td>
</tr>
<tr>
<td>100</td>
<td>0.45</td>
<td>--</td>
</tr>
</tbody>
</table>

* Measured gravimetrically.
Table 8. Change of soil water storage, partitioned for different soil layers, under two different crops.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Plant 1</th>
<th>Plant 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔS(cm/10cm)</td>
<td>S(%)</td>
</tr>
<tr>
<td>10</td>
<td>1.00</td>
<td>26.31</td>
</tr>
<tr>
<td>20</td>
<td>1.10</td>
<td>28.95</td>
</tr>
<tr>
<td>30</td>
<td>0.80</td>
<td>21.05</td>
</tr>
<tr>
<td>40</td>
<td>0.50</td>
<td>13.16</td>
</tr>
<tr>
<td>50</td>
<td>0.40</td>
<td>10.53</td>
</tr>
<tr>
<td>60</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>70</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>80</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>90</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Total</td>
<td>3.80</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Figure 10 compares soil water distribution profiles of the two plant species. Depth of soil water depletion under plant no. 2 is deeper than for plant no. 1. More than 50% of total water depletion, under plant 1, was observed within the first 20 cm of soil layer (Fig. 11). This implies that the roots of plant no. 1 were more active at shallower depths as compared to plant no. 2. Effective rooting depths were 35 and 45 cm for plant no. 1 and 2, respectively.
Measuring Soil Hydraulic Properties

The accurate calculation of solute and water fluxes in field soils is only possible if good data on hydraulic conductivity is available. Many computer simulation models on changes of soil water status in the plant root zone, leaching of fertilizers and other agrochemicals, waste disposal and pollutant transfer problems in soils need information on unsaturated hydraulic conductivity of soils. Experimental work on plant water consumption also necessitates data on hydraulic conductivity measurements. Hydraulic conductivity of field soils is a very important physical property which influences water infiltration characteristics of soils. In drainage work of wet lands, hydraulic characteristics of soils are also needed.

There are a number of methods for the measurement of soil hydraulic conductivity in the field. There are two main approaches that can be used to calculate hydraulic conductivity. The first approach is that described by Nielsen et al. (1976) and necessitates soil water tension measurements in addition to the rate of soil water content decrease within the soil profile. The following relations can be used to calculate the hydraulic conductivity:

\[ K(\theta) = \frac{L(d\theta/dt)}{(dH/dz)} \]  

where \( dH/dz \) is the hydraulic gradient measured at soil depth \( L \) using tensiometers.
The second approach is simpler and does not require tensiometer measurements. Jones and Wagenet (1984) reviewed and compared several simplified methods for measuring soil hydraulic conductivity. One of the methods tested was that described by Libardi et al. (1980). In this approach, \( K(\Theta) \) was of the form

\[
K(\Theta) = K_o \exp[\beta(\Theta_o - \Theta)]
\]

where \( \beta \) is constant and \( K_o \) and \( \Theta_o \) are the values of \( K \) and \( \Theta \) at saturation, respectively. The values of \( \beta \) and \( K_o \) are to be calculated using the field data and the relation

\[
\ln[Z(d\Theta/dt)] = \beta (\Theta_o - \Theta) + \ln K_o
\]

a semi-log plot of the absolute value of \( Z(d\Theta/dt) \) against \( \Theta_o - \Theta \) gives \( \ln K_o \) from the slope and intercept, respectively.

Exercise 4

A training exercise was carried out in the Agency's laboratory at Seibersdorf to measure unsaturated hydraulic conductivity of the research field. A plot of 5x5 m was selected. An access tube was installed in the center of the plot. Levees of 10 cm height, going all around the square-shaped plot, were prepared. The plot received 15 cm of water which was ponded as 5 cm increments. At the completion of applying the last 5 cm water increment, the soil surface was covered to prevent evaporation (a prerequisite of the method). Soil water content was measured at 20 and 30 cm soil depths over a 4 day (96 h) period. Table 9 gives the data.

Table 9. Changes of soil water content (\( \Theta \)) for hydraulic conductivity measurement.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Soil depth, ( Z ) (cm)</th>
<th>Average ( \Theta )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>0</td>
<td>0.470</td>
<td>0.430</td>
</tr>
<tr>
<td>6</td>
<td>0.420</td>
<td>0.410</td>
</tr>
<tr>
<td>12</td>
<td>0.400</td>
<td>0.360</td>
</tr>
<tr>
<td>24</td>
<td>0.380</td>
<td>0.320</td>
</tr>
<tr>
<td>36</td>
<td>0.300</td>
<td>0.360</td>
</tr>
<tr>
<td>48</td>
<td>0.310</td>
<td>0.280</td>
</tr>
<tr>
<td>60</td>
<td>0.305</td>
<td>0.291</td>
</tr>
<tr>
<td>72</td>
<td>0.292</td>
<td>0.284</td>
</tr>
<tr>
<td>84</td>
<td>0.280</td>
<td>0.284</td>
</tr>
<tr>
<td>96</td>
<td>0.291</td>
<td>0.269</td>
</tr>
</tbody>
</table>
Data in Table 9 are enough to estimate unsaturated hydraulic conductivity which is assumed to be adequately described by equation [13].

Table 10 gives the major steps involved in the calculations. The slope \( d\theta/dt \) given in column (2) of Table 10 is calculated using an exponential curve fitted for the plot of \( \bar{\theta} \) versus \( t \). Figure 12 shows the fitting:

\[
\bar{\theta} = 0.557 t^{-0.15289}
\]
\[
d\theta/dt = -8.51 \times 10^{-2} t^{-1.15289}
\]

Table 10. Basic data for estimation of \( K(0)^* \).

<table>
<thead>
<tr>
<th>( \theta ) (m)</th>
<th>( d\theta/dt ) (h)</th>
<th>( ln{Z(d\theta/dt)} )</th>
<th>( \theta_0 - \theta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.450</td>
<td>--</td>
<td>--</td>
<td>0.000</td>
</tr>
<tr>
<td>0.420</td>
<td>-1.179E-2</td>
<td>-1.128</td>
<td>0.030</td>
</tr>
<tr>
<td>0.380</td>
<td>-4.854E-3</td>
<td>-1.927</td>
<td>0.070</td>
</tr>
<tr>
<td>0.350</td>
<td>-2.183E-3</td>
<td>-2.726</td>
<td>0.100</td>
</tr>
<tr>
<td>0.330</td>
<td>-1.366E-3</td>
<td>-3.193</td>
<td>0.120</td>
</tr>
<tr>
<td>0.295</td>
<td>-9.816E-4</td>
<td>-3.525</td>
<td>0.155</td>
</tr>
<tr>
<td>0.298</td>
<td>-7.590E-4</td>
<td>-3.782</td>
<td>0.152</td>
</tr>
<tr>
<td>0.288</td>
<td>-6.151E-4</td>
<td>-3.993</td>
<td>0.162</td>
</tr>
<tr>
<td>0.282</td>
<td>-5.149E-4</td>
<td>-4.170</td>
<td>0.168</td>
</tr>
<tr>
<td>0.280</td>
<td>-4.415E-4</td>
<td>-4.324</td>
<td>0.170</td>
</tr>
</tbody>
</table>

* \( Z = 30 \) cm
\( \theta_0 = 0.45 \) cm/cm³

FIG. 12. Decrease of mean soil water content (\( \theta \)) with time (\( t \)), within the 40 cm of soil depth.
A plot of the variable \((\theta_o - \theta)\) versus \(\ln (Z(d\theta/dt))\) given in Table 10, should give a straight line described by the equation [14].

\[
\ln (Z(d\theta/dt)) = -21.758(\theta_o - \theta) - 0.472, \; r = -0.992
\]

Therefore, the exponential equation [13] describing unsaturated hydraulic conductivity is

\[
K(\theta) = 0.620\exp[-21.758(0.45 - \theta)].
\]

**Plant Water Consumption Studies**

In plant water consumption studies, water balance approach is the simplest method one can use. Figure (13) describes the main components involved in estimating plant water requirement. Water balance in the plant root zone \(L\) can be described by

\[
I + P - (D + ET) = \pm \Delta S \quad [15]
\]

where \(I\) and \(P\), representing irrigation and precipitation can easily be measured, \(\Delta S\) is change in soil water storage of the plant root zone which can be measured with the neutron moisture gauge; \(D\) and \(ET\) are drainage and evapotranspiration terms respectively. Drainage water \(D\) can be estimated with tensiometer data at the rooting depth \(L\), in addition to assessing changes of soil water storage (De Boodt et al., 1967). Integral of water flux over a
given time period, at the lower end of the plant rooting zone, gives the drainage term in equation [15]. Therefore one can write

\[ D = \int_{0}^{t} q \, dt \]  

[16]

where \( q \) is the water flux which may either be positive or negative, depending on water loss below the rooting zone or water gain from ground water table.

The flux \( q \) can be measured, using the well known Darcy's law

\[ q = -K(\theta) \frac{dH}{dz} \]  

[17]

where \( K(\theta) \) is the unsaturated hydraulic conductivity for the water content \( \theta \) at the lower end of the plant rooting zone, and \( dH/dz \) the gradient of soil water hydraulic head, which is measured with tensiometers placed at different soil depths. Under rainfed agricultural systems in arid and semiarid regions, the following assumptions can be made during certain periods of plant growing season:

\[ I = 0; \; P = 0; \; D = 0 \]

In this case, plant water consumption (i.e. evapotranspiration) is simply

\[ E = -AS \]

Exercise 5

1. Seasonal crop water use

In an experiment conducted in the research fields of the Seibersdorf laboratory in 1985, water use of faba beans (\textit{Vicia faba}) was measured. The experimental data of 5 randomly selected plots are given in Table 11.
Table 11. Raw data for crop-water-use study of *Vicia faba*

<table>
<thead>
<tr>
<th>Site No</th>
<th>3</th>
<th>10</th>
<th>17</th>
<th>24</th>
<th>31</th>
<th>40</th>
<th>46</th>
<th>54</th>
<th>61</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 S*</td>
<td>18.4</td>
<td>20.0</td>
<td>20.9</td>
<td>20.1</td>
<td>24.7</td>
<td>18.4</td>
<td>20.8</td>
<td>23.1</td>
<td>20.8</td>
</tr>
<tr>
<td>q</td>
<td>--</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.06</td>
<td>-0.15</td>
<td>-0.08</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2 S</td>
<td>22.1</td>
<td>22.5</td>
<td>22.1</td>
<td>21.6</td>
<td>28.9</td>
<td>21.0</td>
<td>23.6</td>
<td>25.9</td>
<td>22.6</td>
</tr>
<tr>
<td>q</td>
<td>--</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.09</td>
<td>-0.25</td>
<td>0.0</td>
<td>0.02</td>
<td>0.0</td>
</tr>
<tr>
<td>3 S</td>
<td>19.6</td>
<td>22.3</td>
<td>22.2</td>
<td>20.9</td>
<td>25.6</td>
<td>20.4</td>
<td>23.9</td>
<td>25.5</td>
<td>23.1</td>
</tr>
<tr>
<td>q</td>
<td>--</td>
<td>0.02</td>
<td>0.0</td>
<td>0.0</td>
<td>0.08</td>
<td>-0.08</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>4 S</td>
<td>16.4</td>
<td>18.9</td>
<td>18.7</td>
<td>17.7</td>
<td>23.2</td>
<td>16.7</td>
<td>19.3</td>
<td>21.5</td>
<td>18.7</td>
</tr>
<tr>
<td>q</td>
<td>--</td>
<td>0.03</td>
<td>0.0</td>
<td>-0.01</td>
<td>0.05</td>
<td>-0.12</td>
<td>0.0</td>
<td>0.0</td>
<td>-0.02</td>
</tr>
<tr>
<td>5 S</td>
<td>13.6</td>
<td>16.8</td>
<td>16.3</td>
<td>15.2</td>
<td>21.0</td>
<td>14.5</td>
<td>18.6</td>
<td>19.6</td>
<td>17.0</td>
</tr>
<tr>
<td>q</td>
<td>--</td>
<td>0.01</td>
<td>0.0</td>
<td>0.0</td>
<td>0.06</td>
<td>-0.14</td>
<td>0.05</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

| I(cm)** | 1.0 | 1.4 | 1.4 | 1.4 | 3.0 | 3.0 | 3.0 | 4.2 | 4.2 |
| P(cm)    | 0.0 | 0.4 | 0.9 | 2.8 | 4.9 | 6.4 | 8.8 | 8.8 | 10.0|

* Storage S(cm/100cm of soil depth)
Flux q(cm/d)
** I and P are cumulative values, until the indicated days after planting.

The estimation of overall crop water use of faba beans, during 61 days, is as follows:

Site No......................... : 1
Change of soil water storage ΔS..... : 20.8 - 18.4 cm/100 cm
Total irrigation water applied I(cm) : 4.2
Total rainfall P(cm)..................... : 10.0
Drainage D = ΣqΔt (cm)............. : 7x0.06 - 9x0.15 - 6x0.08
Runoff ......................... : Assumed to be 0 cm.

Using equation [15] gives

4.2 + 10.0 - (-1.41 +ET) = 2.4

ET = 13.2 cm

Complete the calculations for other sites.
2. Crop water use at different growth stages

Calculations are similar for the overall crop water use. Suppose it is desired to compare crop water usage during the first one month of growth with that of the remaining period. Example calculations, again completed for the site no. 1, are as following:

Site No.......................... : 1
First 31 days
Change of soil water storage ΔS.... : 24.7 - 18.4 cm/100 cm
Total irrigation water applied I(cm) : 6.3
Total rainfall P(cm).................. : 3.0
Drainage D = ΣqΔt (cm).............. : 4.9
Runoff.............................. : Assumed to be 0 cm

From equ. [15] ET (cm).............. : 1.2 cm in 31 days

Last 30 days

Substraction of the crop water use in the first growth period from the seasonal crop water use results

\[
ET = 13.2 - 1.2
= 12.0 \text{ cm in 30 days.}
\]

Note that crop-water use increased about 10 times in the second half of the growing period. One can carry out similar measurements for shorter time intervals and examine time and growth stage dependence of crop water usage.

Complete the measurements for other sites and comment on the field variability.

Water Use Efficiency Studies

In arid and semiarid regions, the availability of water resources is often critical, and thus irrigation water is not always available, and consequently dry farming practices are commonly used. Crop cultivars with high water use efficiency (WUE) have prime importance for optimum use of available water resources. Hence research to select crop varieties with high WUE has
immediate practical application. For the estimation of WUE, the essential requirement is an estimate of the crop water consumption during the growing season, which is easily assessed with neutron moisture gauges as described above. The following equation is used to calculate WUE:

\[
WUE = \frac{\text{Yield}}{\text{water consumption}} \quad [18]
\]

The yield can be expressed in various ways, depending on interests of the farmers. In some areas, only grain yield may be important, but in others crop residues can equally be valuable as animal feed. Therefore, the yield \((Y)\) is better expressed as the above-ground dry matter. The above equation ignores the root system although it constitutes a substantial portion of the total plant mass (Brown et al., 1987; Gregory et al., 1984). Total water used at field level \((U)\) can be partitioned into its respective components as

\[
U = E + T + R + D \quad [19]
\]

where \(E\) is evaporation from soil surface, \(T\) is transpiration, \(R\) is runoff and \(D\) is the water lost through deep percolation. In general practice, it is difficult to apportion water use into that lost by transpiration and that lost by soil evaporation, and therefore they are combined and called evapotranspiration \((ET)\).

Combining equation [19] in the relation for WUE gives

\[
WUE = \frac{Y}{U}
\]

\[
WUE = \frac{Y/T}{1 + (E+R+D)/T} \quad [20]
\]

Where the ratio \(Y/T\) is called transpiration efficiency. Copper et al. (1987) discussed various agronomic practices available for increasing crop water use efficiency. It is apparent from equation [20] that there are 3 principal ways to increase WUE (Copper et al., 1987). First, changing transpiration efficiency \((Y/T)\); second, increasing \(T\) by way of increasing total water supply at field level; third, if the supply of water is limited, reducing water loss through pathways other than transpiration. Reader should refer to Copper et al. (1987) for more comprehensive discussion on WUE.
Exercise 6

Water use efficiency of *Vicia faba*

Table 12 gives total-dry yield data of *Vicia faba* for the same plots shown in Table 11. Estimate field variation in WUE of *Vicia faba*, using water-use data provided in Table 1.

Site No...............: 1
WUE.....................: $Y/\text{Total ET}$

$: \frac{2322.7}{13.2} = 175.96 \text{ kg/ha.cm}$

Completion of calculations for other plots is an easy exercise. Discuss what additional data is needed to attribute the observed variability in WUE to field variability alone. What soil and crop properties should have been measured to explain the observed field variability?

Table 12. Total dry-yield data of *Vicia faba*.

<table>
<thead>
<tr>
<th>Site No.</th>
<th>Yield, $Y$ (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2322.7</td>
</tr>
<tr>
<td>2.</td>
<td>2654.2</td>
</tr>
<tr>
<td>3.</td>
<td>2281.6</td>
</tr>
<tr>
<td>4.</td>
<td>2275.6</td>
</tr>
<tr>
<td>5.</td>
<td>2114.6</td>
</tr>
</tbody>
</table>

**Irrigation Scheduling**

It is the usual practice to use available soil water content as a criterion for deciding when irrigation is needed. Soil water content profiles based on measurements made with neutron moisture gauges can describe the depletion of available soil water rather accurately. Although it may vary depending on different type of crops, the usual practice is such that the irrigation is started when available water content is decreased down to 50%.
Exercise 7

Table 13 gives soil water content distribution profiles of a field planted to sorghum. Water retention characteristics of the field soil are as follows:

1. Field capacity.... FC = 0.32 cm$^3$/cm$^3$
2. Wilting point..... WP = 0.12 cm$^3$/cm$^3$

If it is assumed that effective rooting depth of sorghum is 40 cm, soil water storage at field capacity is

$$S(FC) = 0.32 \times 40 = 12.8 \text{ cm/40 cm of soil}$$

Soil water storage should be maintained at this level, if available soil water, for plant growth, is to be kept at the level of 100%. Minimum soil water storage at wilting is

$$S(WP) = 0.12 \times 40 = 4.8 \text{ cm/40 cm of soil}$$

which conversely designates 0 % available water. Total available water (TAW) is

$$TAW = S(FC) - S(WP)$$
$$= 12.8 - 4.8 = 8\text{ cm/40 cm soil.}$$

For site no. 1, calculate soil water storage for the date 86/06/26.

From equation [11]

$$S (\text{cm/40 cm}) = 20 \times 0.223 + 10 \times 0.247 + 10 \times 0.197$$
$$= 8.9 \text{ cm/40cm}$$

Available water (AW) left in the soil is

$$AW = 8.9 - S(WP)$$
$$= 8.9 - 4.8 = 4.1 \text{ cm/40 cm of soil}$$

$$\%AW = (AW/TAW) \times 100$$
$$= (4.1/8.0) \times 100 = 51\%$$

which indicates that AW decreased to just the level which was permissible, and therefore the field has to be irrigated.
The net amount of irrigation water needed to bring the AW to 100 % level is simply the difference between soil water storage, prior to irrigation, and the storage at field capacity, S(FC):

\[ I = S(FC) - S \]

\[ = 12.8 - 8.9 = 3.9 \text{ cm} \]

\[ = 39 \text{ mm or } 390 \text{ m}^3/\text{ha}. \]

Table 13. Water content distributions at randomly selected sites of a sorghum field.

<table>
<thead>
<tr>
<th>Site No</th>
<th>Depth Z (cm)</th>
<th>86/06/26 ( \theta ) (cm(^3)/cm(^3))</th>
<th>86/07/03 ( \theta ) (cm(^3)/cm(^3))</th>
<th>86/07/17 ( \theta ) (cm(^3)/cm(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>0.223</td>
<td>0.179</td>
<td>0.182</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.247</td>
<td>0.195</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.192</td>
<td>0.164</td>
<td>0.129</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.204</td>
<td>0.183</td>
<td>0.192</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.226</td>
<td>0.193</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.192</td>
<td>0.158</td>
<td>0.109</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>0.188</td>
<td>0.165</td>
<td>0.151</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.222</td>
<td>0.182</td>
<td>0.139</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.219</td>
<td>0.180</td>
<td>0.106</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0.215</td>
<td>0.174</td>
<td>0.179</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.233</td>
<td>0.196</td>
<td>0.175</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.257</td>
<td>0.228</td>
<td>0.173</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0.228</td>
<td>0.174</td>
<td>0.162</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.207</td>
<td>0.156</td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.168</td>
<td>0.123</td>
<td>0.089</td>
</tr>
</tbody>
</table>

Complete the calculations for all sites and discuss whether a constant irrigation interval could be estimated.
REFERENCES


