A GUIDE TO THE USE OF NITROGEN-15 AND RADIOISOTOPES IN STUDIES OF PLANT NUTRITION: CALCULATIONS AND INTERPRETATION OF DATA

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FOREWORD

The Joint FAO/IAEA Division of Radiation Applications of Atomic Energy for Food and Agriculture Development of the IAEA frequently has technical responsibility for Training Courses organized by the Agency on the use of isotopes in soil fertility-plant nutrition research. Several workshops of a similar nature have also been held for participants in Agency programmes. This manuscript was compiled from the lectures presented at such courses.

The manuscript was edited by Drs. S.K.A. Danso and J.B. Bole of the Joint FAO/IAEA Division and Dr. F. Zapata of the IAEA Seibersdorf Laboratory. It is based on the lectures given by themselves and by Drs. M. Fried and H. Broeshart of the Joint FAO/IAEA Division and Seibersdorf Laboratory respectively.

Many of the examples of isotope aided field and greenhouse experiments used as exercises to illustrate the calculation of such information are actual experimental data from research conducted by the IAEA Seibersdorf Laboratory in support of the Agency's programmes.
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1. ISOTOPES OF NITROGEN.

Various radioactive and stable isotopes of N are known, their mass numbers varying from 12 to 17 (Table 1). 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 usefulness to agricultural research of very short duration. There are two stable isotopes of N which are, $^{14}$N and $^{15}$N. The isotopic composition of $^{15}$N in the atmosphere is approximately 0.366 % of the total N in the atmosphere, while that of $^{14}$N is about 99.634 %. The more or less constant ratio of $^{14}$N/$^{15}$N in the atmosphere or natural substances makes it possible to use N materials artificially enriched or depleted in $^{15}$N in many studies. Since both $^{15}$N and $^{14}$N are not radioactive, the use of the $^{14}$N/$^{15}$N ratios in research does not involve health risks and hazards, and their stable nature allows experiments involving their use to be followed over long periods. In the past, the major factors which discouraged the use of $^{15}$N in agricultural investigations were: the high cost and maintenance problems associated with the instruments used for $^{14}$N/$^{15}$N ratio analysis, and the high cost of $^{15}$N fertilizers, which were accounted for by the low demand for $^{15}$N. Both of these limitations no longer apply. Mass spectrometers have become more reliable and less expensive Emission spectrometers have been developed which are relatively trouble free. The cost of $^{15}$N is now a minor part of the expenses involved in a field experiment.

### TABLE I. THE ISOTOPES OF NITROGEN

<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 min</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 $^{14}$N/$^{15}$N ratio of the atmosphere = 272 ± 0.3, yielding 0.3663 ± 0.0004 $^{15}$N atom %.
2. **BASIC NITROGEN ISOTOPE TERMINOLOGY AND STOICHIOMETRY.**

2.1. **Natural abundance and atom excess:** Natural abundance refers to the relative amounts of $^{14}\text{N}$ and $^{15}\text{N}$ of samples in nature, while atom excess refers to the difference between the relative amounts of $^{14}\text{N}$ and $^{15}\text{N}$ in a given material and that of natural abundance.

Thus, if $a_o = \%^{15}\text{N}$ natural abundance

($\%^{15}\text{N}$ atom (at.) of nitrogen in nature). This is generally about 0.3663 $\%^{15}\text{N}$ at. or approx. 0.366 $\%^{15}\text{N}$ at.

$a = \text{The total atom } \%^{15}\text{N in sample. It is also known as } \%^{15}\text{N abundance. This is equal to the } \%^{15}\text{N natural abundance in nature, but is higher in }^{15}\text{N} \text{ enriched materials, e.g. }^{15}\text{N enriched fertilizer, plant or soil samples supplied with }^{15}\text{N} \text{ enriched fertilizer and lower in materials deliberately depleted of }^{15}\text{N.}$

Then $a_1 = \%^{15}\text{N at. excess in the material, (i.e. } \%^{15}\text{N at. in excess of natural abundance) or total } \%^{15}\text{N at. in sample} - \%^{15}\text{N natural abundance.}$

$$a_1 = a - a_o \quad (1)$$

**Examples:**

(1) The total $\%^{15}\text{N}$ at. for a plant sample analysed in a mass spectrometer was 0.855. What is the $\%^{15}\text{N}$ at. excess in the plant sample?

(2) A $^{15}\text{N}$ enriched fertilizer purchased contained 5.35$\%^{15}\text{N}$ at. excess. What is the total $\%^{15}\text{N}$ at. in this fertilizer?
Calculations:

(1) Total $^{15}$N at. in sample = 0.855  
$^{15}$N natural abundance in sample = 0.366  
$^{15}$N at. excess = 0.855 - 0.366  
= 0.489  
Thus the plant sample has 0.489 $^{15}$N at. in excess of what occurs in nature.

(2) $^{15}$N at. excess in fertilizer = 5.35  
$^{15}$N natural abundance = 0.366  
Total $^{15}$N at. in the fertilizer = 5.35 + 0.366  
= 5.716

2.2. The effect of isotopic composition on molecular weight (M.W.), molar mass ($M$) and the exact nitrogen content ($W_N$) of a compound.

Differences in the isotopic N composition of a compound alter the molecular weight, molar mass and the exact nitrogen content of the different enrichments. The examples shown below illustrate these effects:

(EXAMPLE 1.)

Calculate the average atomic weight for N ($A_N$), molecular mass ($M$) and exact N content ($W_N$) in the following compounds:

(1) Ordinary $(\text{NH}_4)_{2}\text{SO}_4$  
(ii) $(\text{NH}_4)_{2}\text{SO}_4$ with 5% $^{15}$N abundance  
(iii) $(\text{NH}_4)_{2}\text{SO}_4$ with 10% $^{15}$N abundance  
(iv) $(\text{NH}_4)_{2}\text{SO}_4$ with 50% $^{15}$N abundance.
Solution:

To estimate $A_N$, the following formula is used:

$$A_N = \frac{a \times 15 + (100-a) \times 14}{100} \quad (2)$$

where $a = \% \text{ }^{15}\text{N} \text{ abundance}$

<table>
<thead>
<tr>
<th>Ammonium sulphate, ($\text{NH}_4\text{)}_2\text{SO}_4$</th>
<th>Ordinary</th>
<th>5% $^{15}\text{N} \ast$</th>
<th>10% $^{15}\text{N} \ast$</th>
<th>50% $^{15}\text{N} \ast$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_N$(g)</td>
<td>14.00</td>
<td>14.05</td>
<td>14.1</td>
<td>14.5</td>
</tr>
<tr>
<td>$M$(g/mol)</td>
<td>$28+8+32+64=132$</td>
<td>$28.1+8+32+64=132.1$</td>
<td>$28.2+8+32+64=132.2$</td>
<td>$29+8+32+64=133$</td>
</tr>
<tr>
<td>$W_N(%N)$: $\frac{28 \times 100}{132} = 21.21$</td>
<td>$\frac{28.1 \times 100}{132.1} = 21.27$</td>
<td>$\frac{28.2 \times 100}{132.2} = 21.33$</td>
<td>$\frac{29 \times 100}{133} = 21.8$</td>
<td></td>
</tr>
</tbody>
</table>

\( \ast \) = \% $^{15}\text{N} \text{ abundance}

(EXAMPLE 2.)

How many grams of $^{15}\text{N}$ are there in 100 g of each of the materials listed in the above example?

Solution:

The following equations are used in the calculations:

Number of moles = $\frac{\text{weight of compound}}{\text{mol. weight (M) of compound}} \quad (3)$

Number of N atoms = Number of moles of compound $\times$ Number of atoms of N per mole of compound \quad (4)

Number of $^{15}\text{N}$ atoms = $\frac{\% \text{ }^{15}\text{N} \text{ abundance}}{100} \times$ Number of N atoms \quad (5)

Mass of $^{15}\text{N}$ = Number of $^{15}\text{N}$ atoms $\times$ Atomic weight of the $^{15}\text{N}$ isotope \quad (6)
(a) **Ordinary \((\text{NH}_4)_2\text{SO}_4\)**

Number of moles in 100 g of \((\text{NH}_4)_2\text{SO}_4 = \frac{100}{132} = 0.758\)

But 1 mole of \((\text{NH}_4)_2\text{SO}_4\) contains 2 atoms of N

Therefore the number of N atoms = \(2 \times 0.758 = 1.516\)

For ordinary ammonium sulphate, having a % \(^{15}\text{N}\) natural abundance of 0.366,

No. of at. of \(^{15}\text{N}\) = \(0.366 \times 1.516 = 0.00555\)

and mass of \(^{15}\text{N}\) = \(0.00555 \times 15 = 0.0832\) g \(^{15}\text{N}\)

(b) \((\text{NH}_4)_2\text{SO}_4\) 5% \(^{15}\text{N}\) abundance.

Number of moles for 100 g material = \(\frac{100}{132.1} = 0.757\) and number of N atoms = \(2 \times 0.757 = 1.514\).

Therefore, 5 % \(^{15}\text{N}\) abundance \((\text{NH}_4)_2\text{SO}_4\) contains

\(\frac{5}{100} \times 1.514 = 0.0757\) at. \(^{15}\text{N}\)

and \(0.0757 \times 15 = 1.136\) g \(^{15}\text{N}\).

(c) \((\text{NH}_4)_2\text{SO}_4\) 10% \(^{15}\text{N}\) abundance.

Number of moles = \(\frac{100}{132.2} = 0.756\) and atoms N = \(2 \times 0.756 = 1.513\)

Therefore 10% \(^{15}\text{N}\) abundance \((\text{NH}_4)_2\text{SO}_4\) contains

\(\frac{10}{100} \times 1.513 \text{ at.} = 0.1513\) at. \(^{15}\text{N}\)

and \(0.1513 \times 15 = 2.27\) g \(^{15}\text{N}\)

(d) \((\text{NH}_4)_2\text{SO}_4\) 50% \(^{15}\text{N}\) abundance.

Number of moles = \(\frac{100}{133} = 0.752\) and \(2 \times 0.752 = 1.503\) at. N
Therefore 50% $^{15}\text{N}$ abundance $(\text{NH}_4)_2\text{SO}_4$ contains
\[
\frac{50 \times 1.503}{100} = 0.7515 \text{ at. } ^{15}\text{N}
\]
and $0.7515 \times 15 = 11.27 \text{ g } ^{15}\text{N}$.

From the above calculations (Page 8), it has been shown that the molecular weight, and $^\%$N content of a given fertilizer material vary, depending on the $^{15}\text{N}$ enrichment. Also, the $^{15}\text{N}$ contents of fertilizers with $^{15}\text{N}$ greater than natural abundance ($^{15}\text{N}$ enriched fertilizers) are underestimated if the calculations made are based on the $\text{N}$ content of the ordinary or unlabelled fertilizer. The example below, illustrates the latter point.

2.3. Underestimation of $^{15}\text{N}$ content of $^{15}\text{N}$ enriched materials

By how much do you underestimate the $^{15}\text{N}$ content in an ammonium sulphate fertilizer, containing 50% $^{15}\text{N}$ abundance if the calculation is wrongly based on the $^\%$N content of ordinary $(\text{NH}_4)_2\text{SO}_4$?

Solution:

If one wrongly assumes that the $^\%$N content of the enriched material is the same as ordinary $(\text{NH}_4)_2\text{SO}_4$, which is 21.21 g N per 100 g, then the mass of $^{15}\text{N}$ in ammonium sulphate containing 50% $^{15}\text{N}$ abundance is
\[
\frac{50 \times 21.21 \text{ g } ^{15}\text{N}}{100} = 10.6 \text{ g } ^{15}\text{N}
\]

However, from the previous calculations based on the molar masses (see page 10), it is known that 100 g of $(\text{NH}_4)_2\text{SO}_4$ with 50% $^{15}\text{N}$ abundance contains 11.3 g $^{15}\text{N}$.

Therefore, based on the wrong assumption, $^{15}\text{N}$ is underestimated by
\[
\frac{11.3 - 10.6}{11.3} = 0.7 \text{ g or } \frac{0.7 \times 100}{11.3} = 6.2 \% \]
2.4. Ordering of fertilizers

Based on the knowledge gained from the previous sections, and the examples given below, the amounts paid for the same unit of $^{15}\text{N}$ can be different, depending on whether the quotation is given in terms of $\% \ ^{15}\text{N}$ abundance or $\% \ ^{15}\text{N}$ at. excess.

Example -

Two firms have submitted a bid for 100 g N in the form of $(\text{NH}_4)_2\text{SO}_4$ of 1% and 5% $^{15}\text{N}$ at. excess.

Firm I offers $^{15}\text{N}$ at US$ 60/g $^{15}\text{N}$ at. excess contained in the compound.

Firm II offers $^{15}\text{N}$ at US$ 50/g $^{15}\text{N}$ in the material.

Which of these offers is the cheaper?

Solution -

a) For the 1% $^{15}\text{N}$ at. excess material,

Firm I

Quantity of $^{15}\text{N}$ in excess of natural abundance contained in 100 g N as $(\text{NH}_4)_2\text{SO}_4$ enriched with 1% $^{15}\text{N}$ at. excess

= 1 g $^{15}\text{N}$ at. excess

The quotation is given per g atom excess, therefore the cost = 1 x 60 = US$60.00

Firm II

Quantity of $^{15}\text{N}$ in excess of natural abundance contained in 100 g N as $(\text{NH}_4)_2\text{SO}_4$ with 1% $^{15}\text{N}$ at. excess

= 1 g $^{15}\text{N}$ at. excess

The natural abundance of $^{15}\text{N}$ in 100 g N as $(\text{NH}_4)_2\text{SO}_4$ = \frac{0.37}{100} \times 100 = 0.37 \text{ g }^{15}\text{N}$
Therefore total $^{15}$N abundance in this material = $1 + 0.37 = 1.37 \text{ g } ^{15}\text{N}$
and the cost of the material = $1.37 \times 50 = \text{ US$ 68.50}$

b) For the 5% $^{15}$N at. excess material,

**Firm I**

Quantity of $^{15}$N in excess of natural abundance contained in 100 g N as (NH$_4$)$_2$SO$_4$, enriched with 5 % $^{15}$N at. excess

$^{15}$N at. excess = $\frac{5 \times 100}{100} = 5 \text{ g } ^{15}\text{N}$

Since quotation is already based on g $^{15}$N at. excess,

The cost of this compound = $5 \times 60 = \text{ US$ 300}$.

**Firm II**

Quantity of $^{15}$N in excess of natural abundance contained in 100 g N as (NH$_4$)$_2$SO$_4$ with 5% $^{15}$N at. excess = $5 \text{ g } ^{15}\text{N}$

The natural abundance of $^{15}$N in 100 g (NH$_4$)$_2$SO$_4$ = $\frac{0.37}{100} \times 100 = 0.37 \text{ g}$

Therefore total $^{15}$N abundance in the fertilizer = $5 + 0.37 = 5.37 \text{ g}$

Thus cost of this material = $5.37 \times 50 = \text{ US$ 268}$

From the above, it is cheaper to purchase ammonium sulphate from Firm I at the lower enrichment, but at 5% $^{15}$N at. excess, it is more expensive to purchase from Firm I.
3. ISOTOPE DILUTION:

3.1. 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 N fertilizers labelled with different enrichments are mixed:

$$x(a_1) + y(a_2) = (x + y) \overline{a}$$  \hspace{1cm} (1)

Where $x =$ quantity of material with $^{15}$N abundance $a_1$ (of higher $^{15}$N enrichment)

$y =$ quantity of material with $^{15}$N abundance $a_2$ (of lower enrichment)

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

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

$$a_2 = a_o = 0.37 \% \text{ at. } ^{15}\text{N abundance} = 0 \% \text{ at. } ^{15}\text{N at. excess}$$

Equation 1 then becomes

$$\frac{x(a'_1)}{x + y} = \overline{a}$$  \hspace{1cm} (2)

Where $a'_1 =$ % $^{15}$N at. excess of material of higher $^{15}$N enrichment

$\overline{a}' =$ % $^{15}$N at. excess desired in the final mixture.

From an earlier section, it was shown that forms of a compound which differ substantially only in their $^{15}$N enrichment have differences in molecular masses or molecular weights. However, these differences tend to be small with materials whose enrichments are similar. In the latter case, $M_1 \approx M_2,$ (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:

$$m_1 = \frac{(m_1 + m_2) \overline{a}'}{a'_1}$$  \hspace{1cm} (3)

Where the enrichments are very different from each other, $M_1 \neq M_2,$ and 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:

$$\overline{a}' = \frac{n_1 (a'_1)}{n_1 + n_2}$$  \hspace{1cm} (4)

or

$$\overline{a}' = \frac{m_1 (a'_1)M_2}{m_1M_2 + m_2M_1}$$  \hspace{1cm} (5)
and \[ m_1 = \frac{(m_1 + m_2) M_1 a'}{M_2 a' + (M_1 - M_2) a'} \quad (6) \]

3.2. **EXAMPLES**: Making solutions of required \(^{15}\)N content.

(Example 1). From a stock of 2.4% \(^{15}\)N abundance \((\text{NH}_4)_2\text{SO}_4\) and ordinary \((\text{NH}_4)_2\text{SO}_4\), make up a 10 liter solution containing 140 g \((\text{NH}_4)_2\text{SO}_4\) at 1.2% \(^{15}\)N abundance.

**Calculations:**

Let us assume first case, where \(M_1 = M_2\) (i.e. there is no appreciable difference in enrichments).

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

\(2.4\% \text{} ^{15}\text{N} \text{ abundance} = 2.4 - 0.37 = 2.03\% \text{ } ^{15}\text{N} \text{ at. excess} = a'\)

\(1.2\% \text{ } ^{15}\text{N} \text{ abundance desired} = 1.2 - 0.37 = 0.83\% \text{ } ^{15}\text{N} \text{ at. excess} = \overline{a}'\)

Then, \(m_1 = 140 \times 0.83 = 57.2 \text{ g (NH}_4)_2\text{SO}_4\), 2.4\% \(^{15}\)N abundance.

and

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

(Example 2). There are 8 plots, each covering an area of 4 \(m^2\), to be fertilized at a rate of 100 kg N/ha in the form of urea with 1% \(^{15}\)N at. excess. The fertilizers available are urea with 10% \(^{15}\)N abundance and ordinary urea. The fertilizer is to be applied as a solution, each plot receiving 200 ml/m\(^2\). How do you make up the appropriate solution?

**Calculations:**

- **Total N requirement:**
  
  Total area to receive N fertilizer = 8 x 4 (m\(^2\)) = 32 m\(^2\)

  \(100 \text{ kg N/ha} = \frac{100 \times 10^3 \text{ (g) N}}{10^4 \text{ (m}^2\text{)}} = 10 \text{ g N/m}^2\)

  N content of urea = 46.7%

  Amount of urea needed per \(m^2\) = \(\frac{100 \times 10}{46.7} = 21.41 \text{ g}\)

  Amount of urea needed for total area = 21.4 \times 32 = 685.2 \text{ g}
Total volume of solution needed:

\[ 200 \text{ (ml/m}^2) \times 32 \text{ (m}^2) = 6,400 \text{ ml or 6.4 liters.} \] However, to allow for spillage, taking solutions for standards, etc., prepare a total of 6.6 liters. In this case, the amount of urea required is higher by the following ratio \( \frac{6.6}{6.4} \),

thus the required amount for 6.6 liters = \( \frac{6.6 \times 685.2}{6.4} \) = 706.61 g

Dilution calculations:

Since \( M_1 \approx M_2 \),

\[ m_1 + m_2 = 707 \text{ g} \]

\[ a' = 10 - 0.37 = 9.63\% 15N \text{ at. excess of urea to be diluted} \]

\[ a' = 1\% 15N \text{ at. excess desired in final solution} \]

\[ m_1 = 707 \times \frac{1.00}{9.63} = 73.4 \text{ g} \]

\[ m_2 = 707 - 73.4 = 633.60 \text{ g} \]

The required solution is thus obtained by mixing 73.4 g of 10\% 15N abundance labelled urea and 633.6 g of normal urea in 6.6 liters of water.

(Example 3). In a field experiment on nitrogen fixation, 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 to the reference or non fixing crop. The legume will receive 20 kg N/ha applied as 5\% 15N at. excess ammonium sulphate, while the reference crop receives 100 kg N/ha of 1\% 15N at. excess ammonium sulphate. The available fertilizer stock consists of ammonium sulphate with 50\% 15N abundance and unlabelled ammonium sulphate.

The plot sizes are 3.6 m² for the legume, and 1.8 m² for the reference crop. How would you prepare these solutions?
Calculations:

(1) **Legume crop:**

Fertilizer requirement:

Area to be fertilized = 18 \times 3.6 (m^2) = 64.8 m^2

Rate of N application = 20 kg N/ha = 2 g N/m^2

Total N required = 64.8 (m^2) \times 2 (g/m^2) = 129.6 g N

Amount of ammonium sulphate required = \frac{100}{21.2} \times 129.6 = 611 g

Preparation of solution:

Volume required per plot = 200 (ml/m^2) \times 3.6 (m^2) = 720 ml

Total solution needed for 18 plots = 720 \times 18 = 12960 ml

To allow for spillage, etc., prepare 14 liters of solution.

Amount of \((NH_4)_2SO_4\) required for 14 liters = \frac{14}{12.96} \times 611 g = 660 g

Dilutions:

\[ M_1 = 133 \text{ g/mol ammonium sulphate 50\% }^{15}N \text{ abundance} \]

\[ M_2 = 132 \text{ g/mol ordinary ammonium sulphate} \]

\[ m_1 + m_2 = 660 \text{ g} \]

\[ \bar{a}' = 5\% \text{ }^{15}N \text{ at. excess} \]

\[ a'_1 = 50 - 0.37 = 49.63 \text{ }^{15}N \text{ at. excess.} \]

Then:

\[ m_1 = \frac{660 \times 133 \times 5}{(132 \times 49.63) + (133-132)5} = \frac{438900}{6556.1} \text{ or 66.94} \]

Therefore,

\[ m_1 = 66.94 \text{ or approximately 67 g 50\% }^{15}N \text{ abundance } (NH_4)_2SO_4 \]

And, \[ m_2 = 660 - 67 = 593 \text{ g ordinary } (NH_4)_2SO_4 \]

For this experiment therefore, 67 g of 50\% \text{ }^{15}N \text{ abundance ammonium sulphate and 593 g of ordinary ammonium sulphate fertilizer should be dissolved in 14 liters of water, and applied to the legume plots at a rate of 200 ml/m}^2, or 720 ml per plot of 3.6 m^2.
(ii) Standard non-fixing crop:

Fertilizer Requirements:

Area to be fertilized: \(6 \times 1.8 \, (m^2) = 10.8 \, m^2\)

Rate of N application = 100 kg N/ha = 10 g N/m\(^2\)

Total N required = 10.8 \(m^2\) \times 10 (g/m\(^2\)) = 108 g N.

N fertilizer to be used is ammonium sulphate, 21.2% N.

Amount of fertilizer needed = \(\frac{100}{21.2} \times 108 = 509.4 \, g \, ammonium \, sulphate\)

Preparation of solution:

Volume per plot = 200 (ml/m\(^2\)) \times 1.8 \, m^2 = 360 \, ml

Therefore, 6 plots \times 360 (ml/plot) = 2160 ml

Prepare 2.5 liters, to allow for spillage, etc., and also for aliquots of standard to be taken.

This makes the required quantity of fertilizer = \(\frac{2.5}{2.16} \times 509.4 = 590g\)

Dilutions:

\(M_1 = 133 \, g/mol. \, ammonium \, sulphate \, 50\% \, ^{15}N \, abundance,\)

\(M_2 = 132 \, g/mol. \, ordinary \, ammonium \, sulphate,\)

\(m_1 + m_2 = 590 \, g,\)

\(a' = 1\% \, ^{15}N \, at. \, excess,\)

And, \(a' = 50 - 0.37 = 49.63\% \, ^{15}N \, at. \, excess.\)

Then: \(m_1 = \frac{590 \times 133 \times 1}{(132 \times 49.63) + (133 - 132) \, 1}\)

\(m_1 = 78470 = 12 \, g \, 50\% \, ^{15}N \, abundance \, ammonium \, sulphate\)

6552

\(m_2 = 590 - 12 = 578 \, g \, of \, ordinary \, ammonium \, sulphate\)

Therefore, the 1% \(^{15}N \, at. \, excess \, labelled \, ammonium \, sulphate \, solution\)

would be prepared by dissolving 12 g of 50% \(^{15}N \, abundance \, (NH_4)_2SO_4\)

and 578 g of ordinary ammonium sulphate in 2.5 liters of water.
REFERENCES:


4. ISOTOPE USE IN DETERMINING FERTILIZER USE EFFICIENCY

4.1. Basic Concepts

Isotopes provide the only direct method for measuring the amount or proportion of a given nutrient in a fertilizer which is taken up by a plant. They can be used to determine, for example, the efficiency with which plants take up different forms of a fertilizer, such as N in urea versus that in ammonium sulphate, or how different fertilizers and/or cultural practices affect the uptake of a given nutrient. The fertilizer must be labelled with an isotope of the element of interest, but the labelling should not affect the physical and chemical characteristics of this fertilizer. Thus, both the isotopically labelled product and the non-labelled (commercial or normal) fertilizer would behave identically with respect to their availability for plant uptake. The extent to which the isotope is detected in the plant sample gives a measure of the proportion of the nutrient taken up from the fertilizer.

A commonly used measure of the effectiveness of a fertilizer or practice is the fraction or percentage of a particular nutrient derived from an applied isotopically labelled fertilizer.

For phosphorus (P), the parameter is the fraction of the phosphorus in the plant derived from the fertilizer (fPdfF) and is described by the following equations:

\[ f_{PDFF} = \frac{\text{specific activity of plant sample}}{\text{specific activity of the fertilizer applied}} \]  \hspace{1cm} (1)

And, \[ % \text{PdfF} = \frac{\text{specific activity of plant sample}}{\text{specific activity of fertilizer}} \times 100 \]  \hspace{1cm} (2)

Should there be a situation where the plant took up 100% or all its P from the fertilizer and none from any other source (e.g. in pure sand culture), then the specific activity of the plant sample and the fertilizer would be equal.
If half of the P was derived from fertilizer P and half from soil, the specific activity of the plant sample would be half that of the fertilizer supplied. Determining the total amount of the element present in the plant (by routine means of analysis) and multiplying by the % or fraction of PdF, gives the quantity of the P that was taken up from the fertilizer.

Thus, if total P uptake = 40 kg/ha
and \( \% \text{ PdF} = 20 \),

Then quantity of P in plant derived from fertilizer = \( \frac{20}{100} \times 40 \text{ kg/ha} = 8 \text{ kg P/ha} \)

If the only sources of P available to the plant were soil and fertilizer, then the quantity of P in plant derived from soil is 40 - 8 = 32 kg/ha.

The efficiency of utilization of the applied fertilizer can be calculated since the amount applied is known. Thus, if 50 kg P/ha was applied in this example,

\[ \% \text{ utilization of applied P} = \frac{8}{50} \times 100 = 16 \]

For nitrogen, the calculations are essentially the same, except that \( ^{15} \text{N enrichment (}\% \ ^{15} \text{N at. excess)} \) is used here in place of the specific activity used for P and other radioisotopes.

Thus,

\[ f_{\text{NdfF}} = \frac{\% ^{15} \text{N at. excess in plant sample}}{\% ^{15} \text{N at. excess in fertilizer}} \]  

and

\[ \% \text{NdfF} = \frac{\% ^{15} \text{N at. excess in sample}}{\% ^{15} \text{N at. excess in fertilizer}} \times 100 \]
4.2. **Examples:**

4.2.1. **Greenhouse Experiment.**

The analysis of plant samples from a pot experiment showed a % $^{15}$N abundance of 0.8. The fertilizer applied had a % $^{15}$N abundance of 1.6. Calculate (i) what fraction of N was derived from fertilizer, and what fraction was absorbed from soil; (ii) how much of the fertilizer was utilized by the plant, given the following data:

- Each pot contained 2 kg soil
- The N rate was 100 ppm
- Dry matter yield was 15 g
- Plant N content was 2%.

**SOLUTION:**

(i)

% $^{15}$N at. excess in fertilizer = 1.6 - 0.37 = 1.23

% $^{15}$N at. excess in plant = 0.8 - 0.37 = 0.43
Fraction of N derived from fertilizer or \( f_{NdfF} = \frac{0.43}{1.23} = 0.35 \)

or \( \% \ N_{dfF} = \frac{0.43}{1.23} \times 100 = 35\% \)

Since the only sources of N available were soil and fertilizer, the fraction of N derived from soil \( (f_{NdfS}) \) plus fraction from fertilizer \( (f_{NdfF}) = 1 \)

Therefore, \( f_{NdfS} = 1 - 0.35 = 0.65 \) or \( \% \ N_{dfS} = 65\% \).

(ii)

Total N yield = \( \frac{2}{100} \times 15 \text{ g} = 0.3 \text{ g N} \)

N fertilizer yield = \( \frac{35}{100} \times 0.3 = 0.105 \text{ g} \)

The amount of N added to each pot = \( 100 \text{ (mg N/kg)} \times 2 \text{ (kg)} = 200 \text{ mg N} \) or \( 0.2 \text{ g N} \)

Therefore, % of the fertilizer utilized = \( \frac{0.105}{0.2} \times 100 = 52.5\% \)

4.2.2. Field Experiment with maize to find the most efficient method of fertilizer placement.

Treatments - N banded was compared to surface-applied fertilizer, at a rate of 120 kg N/ha as ammonium sulphate, 1.2% \(^{15}\)N abundance.
Each plot consisted of 5 rows of 3 m length and 1 m row spacing.

The harvest area was 3 central rows of 2 m length and 1 m row spacing.

RESULTS - The following primary data were obtained from the plant samples:

<table>
<thead>
<tr>
<th></th>
<th>Banding</th>
<th>Surface broadcast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter yield</td>
<td>3.6 kg</td>
<td>3.4 kg</td>
</tr>
<tr>
<td>(per area harvested)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% N</td>
<td>2.4</td>
<td>2.2</td>
</tr>
<tr>
<td>% (^{15})N abundance</td>
<td>0.91</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Question: Which of the two treatments resulted in a more efficient uptake of the applied N-fertilizer?

Calculation -

a) \( \%NdFF \) (\% N in plant derived from fertilizer)

\[
\% ^{15}\text{N at. excess fertilizer} = 1.2 - 0.37 = 0.83\%
\]

\[
\% ^{15}\text{N at. excess in banded treatment} = 0.91 - 0.37 = 0.54\%
\]
% ^15_15 N at. excess in surface-applied treatment

\[ = 0.75 - 0.37 = 0.38\% \]

% NdfF in banding treatment

\[ = \frac{0.54}{0.83} \times 100 = 65\% \]

% NdfF in surface application

\[ = \frac{0.38}{0.83} \times 100 = 46\% \]

b) N yield per area harvested.

For banding treatment

\[ = 3.6 \text{ kg} \times \frac{2.4}{100} = 0.0864 \text{ kg N} = 86 \text{ g N} \]

For surface application

\[ = 3.4 \text{ kg} \times \frac{2.2}{100} = 0.0748 \text{ kg N} = 75 \text{ g N} \]

c) N fertilizer yield per area harvested.

Banding

\[ = 86 \text{ g} \times \frac{65}{100} \text{ N} = 55.9 \text{ g fertilizer N} \]

Surface

\[ = 75 \text{ g} \times \frac{46}{100} \text{ N} = 34.5 \text{ g fertilizer N} \]

d) % Fertilizer N utilization.

120 kg N/ha

\[ = 12 \text{ g N/m}^2 \]

Area harvested

\[ = 6 \text{ m}^2 \]

Therefore amount applied per area harvested

\[ = 12 (\text{g N/m}^2) \times 6 (\text{m}^2) = 72 \text{ g N} \]

For banding treatment,

% utilization

\[ = \frac{56}{72} \times 100 = 78 \]

For surface treatment,

% utilization

\[ = \frac{34.5}{72} \times 100 = 48 \]

Banding N to maize in this case is superior to surface broadcasting.

4.3. Fertilizer uptake studies without interaction.

It is possible to label two different sources of a particular nutrient element when these are present in one chemical compound. The use of such a compound allows studies of the availability of the different
sources of the element to be made, without any interaction attributable to the presence of nutrients in one compound not being present in the other.

An example is ammonium nitrate, in which both the NH$_4^+$ and NO$_3^-$ ions of NH$_4$NO$_3$ serve as sources of N for plant growth. In an experiment to be described below, the labelling is either on NH$_4^+$ or NO$_3^-$ and thus affords the opportunity to assess their availabilities without interaction.

(Example)

The availability of ammonium and nitrate N to flooded rice was compared in a greenhouse study, using ammonium nitrate fertilizer.

Basic information:

In treatment I, NH$_4$ was labelled, i.e. NH$_4$NO$_3$, with a $\%$ $^{15}$N abundance of 1.39.

In treatment II, NO$_3^-$ was labelled, i.e. NH$_4$NO$_3^*$ with a $\%$ $^{15}$N abundance of 1.52.

Rate of N application = 200 ppm

Weight of soil in each pot = 2 kg.

Experimental results:

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH$_4$NO$_3$</td>
<td>NH$_4$NO$_3^*$</td>
</tr>
<tr>
<td>$%$ $^{15}$N abundance of fertilizer</td>
<td>1.39</td>
<td>1.52</td>
</tr>
<tr>
<td>Dry matter yield</td>
<td>14 g</td>
<td>16 g</td>
</tr>
<tr>
<td>$%$ N content</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td>$%$ $^{15}$N abundance in plant samples</td>
<td>0.70</td>
<td>0.50</td>
</tr>
</tbody>
</table>
Exercise: Calculate 1) the % nitrogen derived from ammonium (%Nf\text{NH}_4) and from nitrate (%Nf\text{NO}_3), 2) the yield of N fertilizer, and 3) the % utilization by the plant of each source of N.

(1) Percent nitrogen derived from each N source

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%15 N at. excess fert. = 1.39-0.37</td>
<td>%15 N at. excess fert. = 1.52-0.37</td>
</tr>
<tr>
<td></td>
<td>= 1.02</td>
<td>= 1.15</td>
</tr>
<tr>
<td></td>
<td>%15 N at. excess plant = 0.70-0.37</td>
<td>%15 N at. excess plant = 0.50-0.37</td>
</tr>
<tr>
<td></td>
<td>= 0.33</td>
<td>= 0.13</td>
</tr>
<tr>
<td></td>
<td>%Nf\text{NH}_4 = 0.33 \times \frac{1.02}{100}</td>
<td>%Nf\text{NO}_3 = 0.13 \times \frac{1.15}{100}</td>
</tr>
<tr>
<td></td>
<td>= 32.4</td>
<td>= 11.3</td>
</tr>
</tbody>
</table>

(2) Yield of N fertilizer source

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N yield = 14 \times \frac{2.2}{100} = 0.308 g</td>
<td>N yield = 16 \times \frac{2.1}{100} = 0.336 g</td>
</tr>
<tr>
<td></td>
<td>N fert. yield = 0.308 \times \frac{32.4}{100}</td>
<td>N fert. yield = 0.336 \times \frac{11.3}{100}</td>
</tr>
<tr>
<td></td>
<td>= 0.1 g</td>
<td>= 0.038 g</td>
</tr>
</tbody>
</table>

(3) % N fertilizer utilization:

The 200 ppm N per pot containing 2 kg soil = 400 mg N per pot.

However, only half, or 50% of the total N of \text{NH}_4\text{NO}_3 corresponds to each source of N.

Therefore, the amount of N in each pot corresponding to each of the two sources is 200 mg or 0.2 g N/pot.

Thus:

\[
\text{% \text{NH}_4 \text{ utilized}} = \frac{0.1}{0.2} \times 100 = 50% \\
\text{% \text{NO}_3 \text{ utilized}} = \frac{0.038}{0.2} \times 100 = 19%
\]

Thus in this study, \text{NH}_4 is a better source of N to rice, since the plant takes up 50% of the amount applied as against only 19% of the \text{NO}_3 source, and the % of N in the plant derived from the \text{NH}_4 and \text{NO}_3 sources are 32.4 and 11.3 respectively.
5. CONCEPT OF AVAILABLE AMOUNTS OF A NUTRIENT TO A CROP.

5.1. Fractional Utilization Ratios

This concept is based on the assumption that, if a plant is confronted with different sources of a nutrient element, the nutrient uptake from each of these sources is proportional to the amounts available from the different sources.

Thus, in a situation where soil and fertilizer are the only sources of nutrient available to a plant:

\[
\frac{\text{Total nutr. in plant}}{\text{Total nutr. supply}} = \frac{\text{Fertilizer nutr. in plant}}{\text{Fertilizer nutr. supply}} = \frac{\text{Soil nutr. in plant}}{\text{Soil nutr. supply}}
\]

or:

\[
\frac{\text{Total nutr. in plant}}{\text{Total nutr. supply}} = \frac{\text{Fertilizer nutr. in plant}}{\text{Fertilizer nutr. supply}} + \frac{\text{Soil nutr. in plant}}{\text{Soil nutr. supply}}
\]

For nitrogen as an example,

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

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

The fertilizer N supply can be considered as the rate of fertilizer N applied. The soil N supply corresponds to the quantity of soil N in 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 the applied fertilizer, for example, in kg N/ha of equivalent units of ammonium sulphate, if this was the fertilizer used.
The total nutrient uptake by any crop which has only soil and fertilizer as sources of nutrient, is the sum of the amounts of the nutrient derived from fertilizer and from soil, all expressed in same units.

For instance in case of nitrogen:

Total N in plant = Fert. N in plant + Soil N in plant

If the total N in plant is expressed in terms of total N yield or uptake (kg N/ha):

Total N yield (kg N/ha) = Fert. N yield (kg N/ha) + Soil N yield (kg N/ha)

But it can also be expressed in relative terms, as fraction or as percentage, such as:

\[ 1 = f_{N df F} + f_{N df S} \]

or \[ 100 = \% N_{df F} + \% N_{df S} \]

Exercise: A field experiment was set up with the objective of assessing the amounts of nitrogen available to a maize crop from soil and applied fertilizer. The maize crop was fertilized with 80 kg N/ha, in the form of ammonium sulphate enriched with 1% \(^{15}\)N at. excess. Determine the fractions of the N which were derived from the fertilizer and from the soil and the availability of soil N relative to the 80 kg/ha fertilizer N based on the following experimental data:

- Dry matter yield = 5000 kg/ha
- Nitrogen content in plant = 2% of the total dry matter yield.
- Isotope content in plant = 0.4% \(^{15}\)N at. excess.

Calculations:
Where the sources of a plant's nutrient supply are only soil and fertilizer, \(f_{N df F} + f_{N df S} = 1\),

\(f_{N df S} = 1 - f_{N df F} \).

But \(f_{N df F} = \frac{\% ^{15}N \text{ at. excess in plant}}{\% ^{15}N \text{ at. excess in fert.}} = \frac{0.4}{1} = 0.4\)

Therefore, \(f_{N df S} = 1 - 0.4 = 0.6\).
From the fractional utilization ratios,

\[ \frac{f_{Ndfs}}{Soil \ N \ supply} = \frac{f_{Ndff}}{Fertilizer \ N \ supply} \]

Therefore,

Soil N supply = Fert. N supply \times \frac{f_{Ndfs}}{f_{Ndff}}

\[ = 80 \times \frac{0.6}{0.4} = 120 \text{ kg N/ha as (NH}_4)_2\text{SO}_4 \]

Total N yield \[ = \frac{\% \ N \ in \ plant}{100} \times \text{D.M. yield} \]

\[ = \frac{2}{100} \times 5000 = 100 \text{ kg N/ha} \]

But from above, \[ f_{Ndff} = \frac{0.4}{1} = 0.4 \]

Therefore N yield from fertilizer \[ = f_{Ndff} \times \text{total N yield} = 0.4 \times 100 \]
\[ = 40 \text{ kg N/ha} \]

But \[ f_{Ndfs} = 1.0 - 0.4 = 0.6 \]

Therefore N yield from soil \[ = f_{Ndfs} \times \text{total N yield} = 0.6 \times 100 \]
\[ = 60 \text{ kg N/ha} \]

And % utilization of added N fertilizer \[ = \frac{40}{80} \times 100 = 50 \]

From the relationship that the amount taken up from each source is directly proportional to the amounts available in each source (fractional utilization constant ratio),

\[ \frac{\text{N yield from fert.}}{A_{\text{fert.}}} = \frac{\text{N yield from soil}}{A_{\text{soil}}} \]

Thus, \[ \frac{40}{80} = \frac{60}{A_{\text{soil}}} \]

Therefore, \[ A_{\text{soil}} = \frac{80 \times 60}{80} = 120 \text{ kg N/ha as (NH}_4)_2\text{SO}_4 \]
This means that the estimated amount of available N in the soil has been found to be equivalent to 120 kg N/ha of applied (NH₄)₂SO₄ under the conditions of the experiment.

5.2. **THE "A-VALUE" CONCEPT**

The soil nutrient supply measured in terms of available units of applied fertilizer N has been referred to as the "A-value". The A-value is based on the concept that when a plant is confronted with two or more different sources of a given nutrient, it will absorb from each in direct proportion to the respective quantities available to that crop.

In a simple case of a plant growing on a soil with an applied labelled fertilizer, the roots can absorb the nutrient in question from only two sources, i.e. the native soil nutrient pool and the known quantity of fertilizer supplied. In this case, it is only necessary to determine the respective quantities absorbed from each source, so as to determine the A-value of soil. Thus, A-value is a yield-independent measure, and can be used to estimate the amount of native soil nutrient available to the plant. This is expressed in fertilizer equivalents. Since the calculation is based on fractional utilization, it follows that:

\[
\frac{\text{Fertilizer nutrient in plant}}{\text{Fertilizer nutrient applied}} = \frac{\text{Soil nutrient in plant}}{\text{Soil nutrient supply (or A-value of soil)}}
\]

and then:

\[
\text{A-value of soil} = \frac{\text{Soil nutrient in plant}}{\text{Fertilizer nutrient in plant}} \times \text{Fertilizer nutrient applied}
\]

Using nitrogen (N) as an example,

\[
\frac{A_N}{fNdfS} = \frac{fNdfS}{fNdfF} \times \text{Rate of fertilizer application}
\]

But

\[
fNdfS = 1 - fNdfF
\]

Thus,

\[
A_N = \frac{1 - fNdfF}{fNdfF} \times \text{Rate of fertilizer application}
\]
In using the A-value concept, it is important to observe the following:

(1) That any change in the nature or the placement method of the labelled fertilizer standard, or in the conditions of plant growth, may affect the magnitude of the A-value for soil.

(2) That the A-value for a particular soil remains constant even at different rates of application of the same labelled fertilizer standard. Thus, in soil fertility studies, it is sufficient to use only one rate of application to assess the nutrient supply potential of a soil.

(3) The A-value of a given soil often changes with time.

A number of exercises below will help to clarify the A-value concept:

5.2.1. **Greenhouse Experiments**

(Exercise 1)

Objective:
To determine the A nitrogen value of a given soil, using barley, oat, ryegrass or any other fast-growing plant

Basic Information:
Fertilizer: 1% $^{15}$N at. excess labelled urea applied at a rate of 80 mg N/pot in pots, each containing 1 kg air-dried soil

Experimental Results:
$\%^{15}$N at. excess in plant sample = 0.25
Calculation:
\[ f_{NdF} = \frac{0.25}{1.00} = 0.25 \]

Therefore \[ f_{NdS} = 1 - 0.25 = 0.75 \]

Thus, \[ \frac{0.25}{80} = \frac{0.75}{A \text{ soil}} \]

And \[ A_N \text{ for the soil} = 80 \times \frac{0.75}{0.25} = 240 \text{ mg N/pot} \]

as equivalent units of urea

(Exercise 2)

Objective:

A pot experiment was carried out to assess the effect of different N rates (in the form of ammonium sulphate) on the supply of nitrogen from various soils

Test crop: barley

Results:

<table>
<thead>
<tr>
<th>N Rate (kg N/ha)</th>
<th>Dry Matter Yield (g/pot)</th>
<th>N Yield from Shoots (mg/pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.42</td>
<td>13</td>
</tr>
<tr>
<td>50</td>
<td>2.08</td>
<td>47</td>
</tr>
<tr>
<td>100</td>
<td>2.09</td>
<td>58</td>
</tr>
<tr>
<td>200</td>
<td>3.06</td>
<td>116</td>
</tr>
</tbody>
</table>

(1) Estimate the amount of nitrogen derived from fertilizer (NdfF as mg/pot)

(2) Calculate nitrogen derived from the soil

(3) Calculate the A-values for the 50, 100 and 200 kg N/ha rates, in kg N/ha as ammonium sulphate equivalent.
A) Based on the "difference in N yield method",

Assume that the N yield of the control gives the amount of nitrogen derived from the soil.

<table>
<thead>
<tr>
<th>N rate (kg N/ha)</th>
<th>N yield (mg/pot)</th>
<th>Ndfsoil (mg/pot)</th>
<th>NdfF* (mg/pot)</th>
<th>Fert. use efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13</td>
<td>13</td>
<td>-</td>
<td>--</td>
</tr>
<tr>
<td>50</td>
<td>47</td>
<td>13</td>
<td>34</td>
<td>68</td>
</tr>
<tr>
<td>100</td>
<td>58</td>
<td>13</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>200</td>
<td>116</td>
<td>13</td>
<td>103</td>
<td>51.5</td>
</tr>
</tbody>
</table>

NdfF* obtained by subtracting N yield of treatment without applied fertilizer from N yield of treatment which received a given amount of fertilizer.

Calculations:

\[
A_N \text{ at } 50 \text{ kg N/ha} = 50 \times \frac{13}{34} = 19 \text{ kg N/ha}
\]

\[
A_N \text{ at } 150 \text{ kg N/ha} = 150 \times \frac{13}{45} = 29 \text{ kg N/ha}
\]

\[
A_N \text{ at } 200 \text{ kg N/ha} = 200 \times \frac{13}{103} = 25 \text{ kg N/ha}
\]

B) Based on isotope method:

<table>
<thead>
<tr>
<th>N Rate (kg N/ha)</th>
<th>N Yield (mg/pot)</th>
<th>Ndfsoil (mg/pot)</th>
<th>NdfF** (mg/pot)</th>
<th>Fertilizer Use efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13</td>
<td>13</td>
<td>-</td>
<td>--</td>
</tr>
<tr>
<td>50</td>
<td>47</td>
<td>33</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>100</td>
<td>58</td>
<td>31</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>200</td>
<td>116</td>
<td>44</td>
<td>72</td>
<td>36</td>
</tr>
</tbody>
</table>

NdfF** = Nitrogen derived from isotope-labelled fertilizer. This value has been experimentally obtained. (Aleksic et. al., 1968, Plant and Soil 29:474).
Calculations:

\[ A_N \text{ at } 50 \text{ kg N/ha} = 50 \times \frac{33}{14} = 118 \text{ kg N/ha} \]

\[ A_N \text{ at } 150 \text{ kg N/ha} = 150 \times \frac{31}{27} = 115 \text{ kg N/ha} \]

\[ A_N \text{ at } 200 \text{ kg N/ha} = 200 \times \frac{44}{72} = 122 \text{ kg N/ha} \]

Note:

1) The experimentally determined NdfF (with isotope) differed appreciably from the estimate made by subtracting the check treatment from the fertilizer treatment, thus the A-values also differed greatly.

2) As the isotope method was directly measuring fertilizer N content of the barley, we can conclude the difference method did not provide an accurate measure of fertilizer N uptake.

3) A-values obtained with the isotope method were not affected by different rates of N application.
5.2.2. Field Experiment

Objective: To compare the available amounts of nitrogen in various \textsuperscript{15}N-labelled urea treatments on winter wheat.

Basic Information:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N Yield (kg/ha)</th>
<th>NdfF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grain</td>
<td>Straw</td>
</tr>
<tr>
<td>1) 100 kg N$^*$/ha urea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall season applied</td>
<td>17.0</td>
<td>4.5</td>
</tr>
<tr>
<td>2) 100 kg N$^*$/ha urea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 kg at tillering stage</td>
<td>14.0</td>
<td>2.8</td>
</tr>
<tr>
<td>50 kg at heading stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) 100 kg N$^*$/ha as 30% S-coated urea - Fall season applied</td>
<td>7.7</td>
<td>1.8</td>
</tr>
<tr>
<td>4) 100 kg N$^*$/ha as 50% S-coated urea - Fall season applied</td>
<td>7.6</td>
<td>1.8</td>
</tr>
<tr>
<td>5) 100 kg N$^*$/ha urea + 2.5% PBQ - Fall season applied</td>
<td>13.0</td>
<td>3.2</td>
</tr>
</tbody>
</table>
Calculated data of the N fertilizer yield, A-values and comparison of the relative efficiencies of the different treatments are given on the following table.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant part</th>
<th>N Yield (kg/ha)</th>
<th>Ndff %</th>
<th>N Fert. Yld (kg/ha)</th>
<th>A-values kg/ha</th>
<th>Efficiency Relative to Treatment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Straw</td>
<td>17.0</td>
<td>37</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Straw</td>
<td>14.0</td>
<td>45</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grain</td>
<td>4.5</td>
<td>38</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grain</td>
<td>2.8</td>
<td>39</td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Grain</td>
<td>16.8</td>
<td>44</td>
<td>7.4</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Straw</td>
<td>7.7</td>
<td>25</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Straw</td>
<td>7.6</td>
<td>8.9</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grain</td>
<td>1.8</td>
<td>8.0</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Grain</td>
<td>9.4</td>
<td>8.7</td>
<td>0.82</td>
<td>1049</td>
<td>1:8.3</td>
</tr>
<tr>
<td></td>
<td>Straw</td>
<td>13.0</td>
<td>44</td>
<td>5.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Grain</td>
<td>3.2</td>
<td>41</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Straw</td>
<td>16.2</td>
<td>43</td>
<td>7.0</td>
<td>133</td>
<td>1:1.05</td>
</tr>
</tbody>
</table>

It must be realized that the A-value is measured as kg/ha of soil N in the units of available amounts of the applied fertilizer. As the available quantities of the soil N pool would not be greatly affected by the N fertilizer application, the A-value serves as an inverse measure of the effectiveness of the fertilizer. Treatment 5 was thus approximately as effective as applying the fertilizer N at tillering and at heading (treatment 2), while fall applied 50% S-coated urea (treatment 4) was ineffective.
5.3. Planning Field Experiments Based on A-value Concepts

Once the concept is well understood, it is possible to make various estimates, such as the approximate amount of fertilizer needed to achieve a desired level of fertilizer uptake, and the $^{15}$N at. excess of a nitrogen fertilizer needed for a study. The following exercises will explain fully how the A value concept can be used to plan field experiments.

5.3.1. Rate of Fertilizer N Application

From the results of a previous experiment, the $A_N$ of a given soil is known to be equivalent to about 400 kg N/ha as ammonium sulphate. In the design of a new experiment, it is desired to have a value of $N_{dfs}$ in the order of 25% with the same fertilizer. What should be the appropriate rate (B) of $(\text{NH}_4)_2\text{SO}_4$ application?

\[
\frac{\% N_{dfs}}{B} = \frac{\% N_{dfs}}{A \text{ soil}} = \frac{100 - \% N_{dfs}}{A \text{ soil}}
\]

Thus \[\frac{25}{B} = \frac{(100-25)}{400} = \frac{75}{400} \]

Therefore \[B = \frac{400 \times 25}{75} = 133 \text{ kg N/ha as } (\text{NH}_4)_2\text{SO}_4\]

5.3.2. Percent Nitrogen derived from Fertilizer

If the $\% N_{dfs}$ from labelled urea ($F_1$) is known to be 12 for a rate of application ($B_1$) equal to 60 kg N/ha, what would have been the $\% N_{dfs}$ ($F_2$) for 120 kg N/ha ($B_2$)?

Since the A-value is not affected by the rate of fertilizer application $B_1$ and $B_2$, it is possible to equate both relationships as follows:

\[A = B_1 \frac{(100-F_1)}{F_1} = B_2 \frac{(100-F_2)}{F_2}\]

Thus, \[60 \times \frac{(100-12)}{12} = 120 \times \frac{(100-F_2)}{F_2}\]

or

\[ 440 = 120 \times \frac{(100-F_2)}{F_2} \]

\[ 440 F_2 = 12000 - 120 F_2 \]

\[ 560 F_2 = 12000 \]

therefore \[ F_2 = \frac{12000}{560} = 21.4\% \]

or we would have expected a \( \% \text{NdfF} \) of about 21 if 120 kg N/ha of urea had been applied.

5.3.3. Necessary \( ^{15} \text{N} \) Enrichment

The following example provides a method to estimate the \( ^{15} \text{N} \) abundance of \( ^{15} \text{N} \)-labelled fertilizer required for field experimentation.

Known parameters:

- \( A_N \) of the soil = 300 kg N/ha as ammonium sulphate equivalents.
- N rate = 100 kg N/ha as ammonium sulphate.

Question: What \( ^{15} \text{N} \) abundance of the ammonium sulphate should be ordered?

a) Estimate of the expected Ndfa.

The available amounts of N to the plant from the two sources available are 300 kg N/ha and 100 kg N/ha from soil and fertilizer respectively. The total N supply is thus 400 kg N/ha in ammonium sulphate equivalent units.

From the fractional utilization equation,

\[
\frac{\text{Total nutrient in plant}}{\text{Total nutrient supply}} = \frac{f\text{Ndfa}}{f\text{ert.supply}} = \frac{f\text{NdfS}}{\text{Soil supply}}
\]

Therefore \[ \frac{1}{400} = \frac{f\text{Ndfa}}{100} \]
And \[ f_{\text{NdfF}} = \frac{100}{400} = 0.25 \]

b) Calculation of the \(^{15}\text{N}\) abundance of the fertilizer.

\[ f_{\text{NdfF}} = \frac{\% \ ^{15}\text{N at. excess in plant}}{\% \ ^{15}\text{N at. excess in fertilizer}} \]

In order to reduce the influence of normal measurement errors associated with \(^{15}\text{N}\) assay, in this case a minimum of 0.5\% \(^{15}\text{N}\) abundance has been decided as required in the plant samples. *

Therefore \(^{15}\text{N}\) at. excess required in plant = 0.5 - 0.37 = 0.13

Thus \[ 0.25 = \frac{0.13}{\% \ ^{15}\text{N at. excess in fertilizer}} \]

Required \(^{15}\text{N}\) at. excess of fertilizer = \[ \frac{0.13}{0.25} = 0.52 \]

Required \(^{15}\text{N}\) abundance of the fertilizer = 0.52 + 0.37 = 0.89

Considering a safety factor, the \(^{15}\text{N}\) abundance of the fertilizer to be ordered should be between 1 and 1.5%.

5.3.4. Using Isotope Sub-plots

One way to reduce the cost of isotope-aided studies in the field is the practice of only applying the labelled fertilizer to small plots (isotope plots). There are other ways of minimizing the size of the treated area when the cost of the labelled material is particularly high. The investigator, however, will be confronted with specific problems when processing the isotope data.

* The required \(^{15}\text{N}\) content in the plant samples will depend on several considerations, including the particular type of instrument, and its sensitivity.
This exercise illustrates the case when a few rows of the isotope plot are labelled.

Assume the following ISOTOPE PLOT LAYOUT.

| Interlines receiving ordinary ammonium sulphate | Interlines receiving labelled ammonium sulphate 1% $^{15}$N at. excess |

After the harvest of the crop, $^{15}$N at. excess in the plant material is 0.2.

**Question 1:** What would have been the $^{15}$N at. excess in the plant material if all interlines would have received the labelled ammonium sulphate with 1% $^{15}$N at. excess?

**Solution:** Since all interlines receive a similar original application of N at the normal rate R,

$^{15}$N at. excess in the plant material when all interlines receive $^{15}$N-labelled fertilizer = $2 \times$ $^{15}$N at. excess in plant material which had only half of rows fertilized with $^{15}$N-labelled fertilizer.
Therefore, \( \% \begin{array}{l}^{15}N \text{ at. excess in plant} = 2 \times 0.2 = 0.4 \end{array} \)
and \( \% \text{NdfF} = \frac{0.4}{1} \times 100 = 40 \)
but \( \frac{40}{R} = \frac{100-40}{A \text{ soil}} \)
Therefore, \( A \text{-soil} = \frac{R \times 60}{40} = 1.5R \)

**Question 2:** What would be the \( \% \begin{array}{l}^{15}N \text{ at. excess in the sample if the} \end{array} \)
available quantity of \( \begin{array}{l}^{15}N \text{-labelled ammonium sulphate} \end{array} \)
would have been applied to all interlines?

**Solution:** All interlines receive a similar application but at half
the normal rate \( R = (1/2R) \).
It is known that the amount of available nitrogen in the soil (\( A \text{-soil} \))
is not affected by the fertilizer application rates \( R \) and \( 1/2 \ R \).

Thus:
\[
A_{\text{soil}} = R \times \frac{(100-40)}{40} = 1/2R \times \frac{(100-2\text{NdfF})}{\%\text{NdfF}}
\]

\( 1.5R = 1/2 \ R \times \frac{(100-2\text{NdfF})}{\%\text{NdfF}} \)

\( 1.5 \times (\%\text{NdfF}) = 50 - 1/2 \times (\%\text{NdfF}) \)

\( 2 \times (\%\text{NdfF}) = 50 \)
\( \% \text{NdfF} = \frac{50}{2} = 25 \)

And \( f\text{NdfF} = 0.25 \)

Since \( f\text{NdfF} = \frac{\% \begin{array}{l}^{15}N \text{ at. excess in plant} \end{array}}{\% \begin{array}{l}^{15}N \text{ at. excess in fertilizer} \end{array}} \)
and the labelled fertilizer was \( 1\% \ \begin{array}{l}^{15}N \text{ at. excess (NH}_4\text{)}_2\text{SO}_4 \),

\( 0.25 = \frac{\% \begin{array}{l}^{15}N \text{ at. excess in plant} \end{array}}{1\% \begin{array}{l}^{15}N \text{ at. excess in fert.} \end{array}} \)

Answer: 0.25\% \begin{array}{l}^{15}N \text{ at. excess in plant material.} \end{array}
6. EVALUATION OF NUTRIENT SOURCES WHICH CANNOT BE DIRECTLY LABELLED WITH ISOTOPES.

Various nutrient sources exist in nature which cannot be labelled directly, or which can only be labelled with great difficulty. Under such circumstances, it is not possible to use isotopes directly to estimate their ability to supply nutrients to the plant by the methods previously outlined. However, by an extension of the previously described methodology, it is possible to estimate their availability.

As an example, there are plants which satisfy their N requirement not only from soil and applied fertilizer, but also from either symbiotic or asymbiotic fixation of gaseous N\(_2\). There are thus, three sources of N in this case, being: soil N, fertilizer N and N\(_2\) gas. Although it is possible to label N\(_2\) gas with \(^{15}\)N, it is not practical to label all the N\(_2\) gas in the atmosphere in a field where such a crop is growing. Similarly, the nutrients in such natural fertilizer products as guano or rock phosphate cannot be uniformly labelled.

6.1. The Availability of Nitrogen in Guano

To assess the available amount of nitrogen in a natural product such as guano, it is necessary to quantify the entire N pool in the presence and absence of a source of the material. The A-value can be used to measure the amount of nutrients available to plants.

Method: The method requires two treatments. The first has a fertilizer source which is labelled with \(^{15}\)N as the standard treatment. The only other nutrient source in this case is unlabelled native soil N. The A-value obtained from this treatment therefore represents only that of the soil. A second treatment consists of the addition of the same labelled N fertilizer source, plus the guano. There are thus three nutrient sources, labelled fertilizer, unlabelled soil and unlabelled guano. From the second treatment therefore, the A-value obtained represents the sum of available soil N, and available guano N measured in terms of labelled fertilizer equivalents. The difference between the A-values of the second and first treatments represents the A-value of guano or the amount of N available for plant uptake in the guano fertilizer.
Example:

Treatment I: To determine the A-value of the soil.

Soil + $^{15}$N-labelled fertilizer, for instance $^{15}$N-Urea applied at a rate of 80 mg N/pot (STANDARD Treatment).

Treatment II: To determine the A-value of the soil plus the guano.

Soil + $^{15}$N labelled fertilizer (urea applied at a rate of 80 mg N/pot) + N-source which cannot be labelled (guano applied at 200 mg N/pot).

Experimental results:

Treatment I: \[\% \text{NdfF} = 20\]

From the fractional utilization equation, \[\frac{20}{80} = \frac{100-20}{A_{\text{soil}}}\]

Therefore \(A_{\text{soil}} = 320\) mg N/pot as urea equivalents.

Treatment II: \[\% \text{NdfF} = 15\]

Thus, \[\frac{15}{80} = \frac{100-15}{A_{\text{soil + guano}}}\]

Therefore, \(A_{\text{soil + guano}} = 453\) mg N/pot

and \(A_{\text{guano}} = A_{(\text{soil + guano})} - A_{\text{soil}} = 453 - 320\)

Therefore \(A_{\text{guano}} = 133\) mg N/pot as urea equivalents.

It follows that:

200 mg N as guano supplies the same amount of N as 133 mg N of urea does. Thus 100 mg N as guano is equivalent to 66.5 mg N as urea.
Therefore, in this example,

urea is \( \frac{100}{66.5} = 1.5 \) times more efficient than guano per unit of N in fertilizer added.

6.2. The Availability of Phosphorus in Rock Phosphate

A similar methodology to that previously described for guano can also be used to determine the availability of P in natural rock phosphates.

(Example): To evaluate in a greenhouse experiment the plant available amounts of P contained in rock phosphates obtained from two different sources. The standard treatment was \(^{32}\text{P}\) labelled ordinary superphosphate (OSP) mixed thoroughly with the soil, with barley as the test crop.

Treatments:

I. Acid soil plus \(^{32}\text{P}\) labelled OSP (125 mg P/pot), as STANDARD, mixed thoroughly with the soil.

II. Acid soil mixed with BAYOVAR ROCK PHOSPHATE (2500 mg P/pot) and the STANDARD.

III. Acid soil mixed with UPPER VOLTA ROCK PHOSPHATE (2500 mg P/pot) and the STANDARD.
Experimental results:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry matter (g/pot)</th>
<th>P yield (mg P/pot)</th>
<th>PdFF (%)</th>
<th>A value* (mg P/pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.68</td>
<td>14.1</td>
<td>42</td>
<td>179 (Soil)</td>
</tr>
<tr>
<td>II</td>
<td>6.40</td>
<td>28.5</td>
<td>16</td>
<td>657 (Soil + BRP)</td>
</tr>
<tr>
<td>III</td>
<td>5.25</td>
<td>16.5</td>
<td>34</td>
<td>248 (Soil + URP)</td>
</tr>
</tbody>
</table>

* A-values are expressed in mg P/pot as equivalent units of $^{32}$P- OSP mixed thoroughly with the soil.

By difference,

$A_{BRP} = A_{soil + BRP} - A_{soil}$

$= 657 - 179$

$= 478$

$A_{URP} = A_{soil + URP} - A_{soil}$

$= 248 - 179$

$= 69$
Evaluation of rock phosphate materials mixed throughout the soil:

<table>
<thead>
<tr>
<th>Rock phosphate</th>
<th>Grade % P</th>
<th>Rate of application mg P/pot</th>
<th>A-values * mg P/pot</th>
<th>Amount in kg equivalent to 1 Kg P as OSP 1 kg OSP **</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAYOVAR ROCK</td>
<td>13.6</td>
<td>2500</td>
<td>478</td>
<td>5.2</td>
</tr>
<tr>
<td>PHOSPHATE</td>
<td></td>
<td></td>
<td></td>
<td>3.3</td>
</tr>
<tr>
<td>UPPER VOLTA ROCK</td>
<td>9.5</td>
<td>2500</td>
<td>69</td>
<td>36</td>
</tr>
<tr>
<td>PHOSPHATE</td>
<td></td>
<td></td>
<td></td>
<td>33</td>
</tr>
</tbody>
</table>

* Expressed in mg P/pot as OSP equivalents.

** a) BAYOVAR RP

\[
2500 \text{ kg P as Bayovar RP} = 478 \text{ kg P as OSP}
\]

\[
x = \frac{2500}{478} = 5.2 \text{ kg P as BAYOVAR RP}
\]

\[
5.2 \text{ kg P as BAYOVAR RP} = 1 \text{ kg P as OSP}
\]

\[
5.2 \times \frac{100}{13.6} = 1 \times \frac{100}{8.7}
\]

\[
38.2 \text{ kg BAYOVAR RP} = 11.5 \text{ kg OSP}
\]

\[
1 \text{ kg OSP} = \frac{38.2}{11.5} \text{ kg BAYOVAR RP}
\]

\[
1 \text{ kg OSP} = 3.3 \text{ kg BAYOVAR RP}
\]
b) UPPER VOLTA RP

\[
2500 \text{ kg P as Upper Volta RP} = 69 \text{ kg P as OSP}
\]

\[
x = \frac{2500}{69} = 36 \text{ kg P as UPPER VOLTA RP}
\]

\[
36 \text{ kg P as UPPER VOLTA RP} = 1 \text{ kg P as OSP}
\]

\[
36 \times \frac{100}{9.5} = 1 \times \frac{100}{8.7}
\]

\[
379 \text{ kg UPPER VOLTA RP} = 11.5 \text{ kg OSP}
\]

\[
1 \text{ kg OSP} = \frac{379}{11.5} \text{ kg UPPER VOLTA RP}
\]

\[
1 \text{ kg OSP} = 33 \text{ kg UPPER VOLTA RP}
\]

Thus, although it is not possible to label rock phosphates uniformly for isotopic studies of their uptake, the use of the indirect labelling technique has demonstrated here shows that the P in Bayovar rock phosphate is more available than that from the Upper Volta. In addition, it has provided a comparison of each of these rock phosphates with OSP, the P in the latter being 5 times more available than that in BRP, while it is 36 times more available than that in URP. This does not take into consideration the residual value of P in the material which could become available for growth of subsequent plants.
7. **THE USE OF THE $^{15}$N METHODOLOGY TO ASSESS NITROGEN FIXATION BY LEGUMES.**

Many legumes, in symbiosis with the appropriate strains of bacteria belonging to the genus *Rhizobium*, are capable of supplementing N derived from soil by converting atmospheric $N_2$ into forms available to the plant. Such a legume crop growing in the presence of nitrogen fertilizer will thus derive its N from soil, fertilizer and the atmosphere. It is, however, impossible to label all the N in an atmosphere over a field crop with $^{15}$N. Using the indirect labelling technique discussed earlier, it is possible to assess how much of the plant's total N supply was derived from atmospheric $N_2$. The method involves the use of a reference crop, (which does not fix $N_2$) to assess the A-value of the soil, or to determine the relative amounts of available soil and applied $^{15}$N labelled fertilizer N available to a crop. The basic assumption is that if the reference is an appropriate standard, it will absorb the soil N and fertilizer N in the same ratio as the legume, thus reflecting the same amount of available N (in fertilizer units) present in the soil to both crops. The A-value obtained for the legume crop would therefore comprise the available amounts from both soil and $N_2$ fixed.

Thus, by applying the A-value concept, we can determine:

- from the reference (non-fixing) crop $A_{soil}$
- from the legume (fixing) crop $A_{soil} + fixation$
- and $A_{fixation} = A_{soil} + fixation - A_{soil}$

The fractional utilization equation for the legume crop is as follows:

$$\frac{\% \text{ NdfF}}{A_{fert}} = \frac{\% \text{ NdfFix}}{A_{fix}} = \frac{\% \text{ NdfS}}{A_{soil}} = \frac{\% \text{ NdfFix} + \% \text{ NdfS}}{A_{fix} + A_{soil}}$$
Thus, if they have the same amounts of soil plus fertilizer N available to them, they will reflect the same amounts of available soil and fertilizer N by absorbing them in the same ratio. By means of this relationship it is possible to calculate $\% N_{df}$, since $\% N_{dfF}$ can be determined experimentally by the use of $^{15}$N labelled fertilizer. $A_{Fert}$ is also known, since it is equivalent to the amount of fertilizer N applied, and $A_{Fix}$ is the difference in the A-values obtained for the fixing and non-fixing treatments.

From an experimental point of view there can be two situations:

1. Where the $N_2$ fixing and non-fixing crops have available to them the same amounts of soil and fertilizer nitrogen. This is the simplest case, where the same amount of a $^{15}$N labelled material is applied to both crops.
2. Where the $N_2$ fixing and non-fixing crops have available to them the same amount of soil N but receive different amounts of fertilizer N. A "starter" amount or a low rate of N fertilizer is applied to the fixing crop to avoid interference with N fixation, but a higher rate may have to be applied to the reference crop to ensure normal growth.

7.1. Method of calculating N fixed when fixing and non-fixing crops receive the same rate of $^{15}$N fertilizer

To illustrate the methodology, consider the following examples:

<table>
<thead>
<tr>
<th>% $^{15}$N at. excess</th>
<th>Fixing crop</th>
<th>Non-fixing crop</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>No fixation</td>
</tr>
<tr>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
<td>100% fixation</td>
</tr>
<tr>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td>50% fixation</td>
</tr>
<tr>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>80% fixation</td>
</tr>
</tbody>
</table>

This relationship is described in the following equations:
\[
\% \text{NdfFix} = (1 - \frac{\% \text{N at. excess legume crop}}{\% \text{N at. excess reference crop}}) \times 100
\]  

(5)

or

\[
\% \text{NdfFix} = (1 - \frac{\% \text{NdfF legume}}{\% \text{NdfF reference crop}}) \times 100
\]  

(6)

\[
\text{N Fixed (kg/ha)} = \frac{\% \text{NdfFix}}{100} \times \text{Total N yield}
\]  

(7)

(Exercise)

Estimate the percentage of symbiotic nitrogen fixation by broadbean and soybean, given the following data: (The crops were grown in soil labelled by incorporating \(^{15}\text{N}\)-labelled plant material into the soil.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% \text{N at. excess}</th>
<th>N yield in kg/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Barley (reference crop)</td>
<td>0.335</td>
<td>90</td>
</tr>
<tr>
<td>2. Nodulated soybean</td>
<td>0.198</td>
<td>160</td>
</tr>
<tr>
<td>3. Broadbean</td>
<td>0.112</td>
<td>200</td>
</tr>
</tbody>
</table>

The above experiment is similar to a case where both the legume and non-legume received the same rate of \(^{15}\text{N}\) fertilizer, and can tap the same amount of available soil N, since they are grown on the same soil. In such a situation, it is not necessary to calculate the A-values for soil and the following equation can be used directly:

\[
\% \text{NdfFix} = (1 - \frac{\% \text{N at. excess in legume}}{\% \text{N at. excess in reference}}) \times 100
\]

For soybean therefore,

\[
\% \text{NdfFix} = (1 - \frac{0.198}{0.335}) \times 100 = 41
\]

\[
\text{N Fixed} = \frac{41}{100} \times 160 \text{ kg N/ha} = 66 \text{ kg N/ha}
\]

For broadbean,

\[
\% \text{NdfFix} = (1 - \frac{0.112}{0.335}) \times 100 = 67
\]

\[
\text{N Fixed} = \frac{67}{100} \times 200 = 134 \text{ kg N/ha}
\]

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7.2. Method of calculating N fixed when fixing and non-fixing crops receive different rates of $^{15}$N fertilizer

In this case, it is necessary to calculate A-value for the non-nodulating crop and that for the nodulating crop. The non-nodulating crop estimates the A-value for soil, while the A-value obtained for the nodulating crop is the sum of the A-values of the soil plus the N fixed. The difference between the two A-values then represents the A-value of fixation in fertilizer equivalent units, which can be converted into the actual amount of N fixed by multiplying this figure by the N utilization efficiency of the applied fertilizer by the legume.

**EXAMPLE:**

I. Nod. crop

$^{15}$N at. excess = 0.2 % in plant

- 20 kg N/ha $(NH_4)_2 SO_4$ with 5 % $^{15}$N at.excess was applied at planting.

- Total N yield = 200 kg N/ha

II. No-nod. crop

$^{15}$N at excess = 0.4 % in plant

- 100 kg N/ha $(NH_4)_2 SO_4$ containing 1 % $^{15}$N at. excess was applied at planting

**Calculations:**

$\% \text{NdfF} = \frac{\% \text{N at. excess in legume plant}}{\% \text{N at. excess in fertilizer}} \times 100 \quad (8)$

Therefore, $\% \text{NdfF} \quad \text{(nod. crop)} = \frac{0.2}{5} \times 100 = 4$

$\% \text{NdfF} \quad \text{(non.-nod. crop)} = \frac{0.4}{1} \times 100 = 40$

$A\text{-value} = \frac{100 - \% \text{NdfF}}{\% \text{NdfF}} \times \text{Rate of fert. N application} \quad (9)$
Therefore,

\[
A\text{-value (nod. crop)} = \frac{100 - 4}{4} \times 20 = 480
\]

And

\[
A\text{-value (non-nod. crop)} = \frac{100 - 40}{40} \times 100 = 150
\]

Thus, \( A_{\text{soil + fix.}} = 480 \text{ kg N/ha as } (\text{NH}_4)_2\text{SO}_4 \text{ equivalents} \)

And \( A_{\text{soil}} = 150 \text{ " } " " " " " " " " \)

Therefore \( A_{\text{fix}} = 330 \text{ kg N/ha as } (\text{NH}_4)_2\text{SO}_4 \text{ " } " " " \)

To determine the actual amount of N fixed, we must multiply \( A_{\text{fix}} \) by the % fertilizer utilization efficiency of the nodulated crop.

\[
\text{Fertilizer N yield} = \frac{\% \text{ NdfF} \times \text{Total N yield}}{100}
\]

\[
= \frac{4 \times 200}{100} = 8 \text{ kg N/ha}
\]

% Fertilizer utilization efficiency \( (\% \text{ F.U.E.}) \)

\[
= \frac{\text{N fert. yield in plant}}{\text{Fertilizer applied}} \times 100
\]

Therefore, % F.U.E.

\[
= \frac{8}{20} \times 100 = 40
\]

Total amount of N fixed (kg/ha)

\[
= \frac{A_{\text{fix}} \times \% \text{ F.U.E. (nod. crop)}}{100}
\]

\[
= \frac{330 \times 40}{100} = 132 \text{ kg N/ha}
\]

and % NdfFix

\[
= \frac{132}{200} \times 100 = 66
\]

Since for a nodulating crop, total N yield = N yield from fertilizer + N yield from soil + N yield from fixation, the amount of N derived by nod. crop from soil (NdfS) can be calculated as follows:

\[
\text{NdfS} = \text{Total N yield} - (\text{NdfF} + \text{NdfFix})
\]

\[
= 200 - (8 + 132) \text{ kg N/ha}
\]

\[
= 60 \text{ kg N/ha.}
\]

Thus, the amounts of N derived by the nodulating crop from fertilizer, soil and fixation are: 8, 60 and 132 kg N/ha respectively.
There are other ways of calculating N fixed, and below is another method for calculating the above data:

For the nodulating crop

\[ \% \text{NdfF} = \frac{15\text{ N at. excess in sample (plant)}}{15\text{ N at. excess in fertilizer}} \times 100 \]  
\[ = 0.2 \times 5 = 4 \]

The fractional utilization relationship for the nodulating crop is the following:

\[ \frac{\% \text{NdfF}}{\% \text{NdfS} + \% \text{NdfFix.}} = \frac{\% \text{NdfS} + \% \text{NdfFix.}}{A_{\text{soil}} + A_{\text{fixed}}} \]  
\[ \text{But} \ % \text{NdfF} + \% \text{NdfS} + \% \text{NdfFix.} = 100 \]

\[ \% \text{NdfS} + \% \text{NdfFix.} = 100 - \% \text{NdfF} \]

Therefore,

\[ \frac{4}{20} = \frac{96}{A_{\text{soil}} + \text{Fix}} \]

\[ A_{\text{soil}} + \text{Fix.} = \frac{96}{4} \times 20 = 480 \text{ kg N/ha as } (\text{NH}_4)_2\text{SO}_4 \text{ equivalents} \]

For the non-nodulating crop

\[ \% \text{NdfF} = \frac{0.4}{1} \times 100 = 40 \]

In this case, since there is no fixation, only soil and fertilizer N are available for this crop, and therefore:

\[ \frac{\% \text{NdfF}}{\% \text{NdfS}} = \frac{100 - \% \text{NdfF}}{A_{\text{soil}}} \]

Therefore,

\[ \frac{40}{100} = \frac{100 - 40}{A_{\text{soil}}} \]

And \[ A_{\text{soil}} = \frac{60}{40} \times 100 = 150 \text{ kg N/ha as } (\text{NH}_4)_2\text{SO}_4 \text{ equiv.} \]

Thus,

\[ A_{\text{fix}} = A_{\text{soil}} + \text{fix} - A_{\text{soil}} = 480 - 150 \]

\[ = 330 \text{ kg N/ha as } (\text{NH}_4)_2\text{SO}_4 \text{ equivalents} \]
Furthermore, it was shown that for the nodulating crop

\[
\frac{4}{20} = \frac{\% \text{ NdfFix}}{330} = \frac{\% \text{ NdfS}}{150}
\]

Therefore \( \% \text{ NdfFix} = \frac{330 \times 4}{20} = 66 \% \)

\( \% \text{ NdfS} = \frac{150 \times 4}{20} = 30 \% \)

And \( \% \text{ NdfF} = 4 \% \)

Total \( = 100 \% \).

Since the total N yield/ha \( = 200 \text{ Kg N/ha} \)

Therefore the amount of N fixed \( = \frac{\% \text{ NdfFix}}{100} \times \text{ N yield} \)

\( = \frac{66}{100} \times 200 = 132 \text{ kg N/ha} \)

The amount of N derived from soil \( = \frac{30}{100} \times 200 = 60 \text{ kg N/ha} \).

The amount of N derived from fert. \( = \frac{4}{100} \times 200 = 8 \text{ kg N/ha} \).
8. SOME BASIC CONCEPTS ON THE USE OF RADIOACTIVE ISOTOPES.

Many of the concepts of stable isotope use are equally useful in agricultural experiments using radioisotopes. In fact, concepts such as the A-value and isotope dilution were developed with radioisotopes. Experiments with radioisotopes are normally less expensive to conduct and radioisotope 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 the IAEA publication "Safe Handling of Radionuclides", 1973 (STI/PUB/319).

All radioisotopes are decaying 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. A radioisotope thus has a characteristic 1) rate constant of decay (λ), and or a half-life T½; 2) decay scheme and type of ionizing radiation (alpha, beta and/or gamma); 3) energy pattern of the particles or rays. These concepts are explained in the FAO/IAEA Tracer Manual on Crops and Soils (STI/DOC/10/171).

8.1 Decay Law

As a radioisotope decays the total number of radioactive atoms present (N) decreases at a rate proportional to N, given by:

\[ \frac{dN}{dt} = -\lambda N \]  

(1)

where \( \frac{dN}{dt} \) is the disintegration or decay rate and the constant (λ) is termed the decay constant. Integration of equation (1) over time yields the exponential decay law:

\[ N = N_0 e^{-\lambda t} \]  

(2)

Decay or desintegration curves of a single radionuclide can be plotted on semilogarithmic paper by using the following relationships:

\[ \ln N = \ln N_0 - \lambda t \]  

(3)

where ln is the natural logarithm or
\[
\log N = \log N_0 - \frac{\lambda}{2.3} t
\]

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

Since the rate of decay \(\frac{dN}{dt}\) can be also expressed in terms of an activity \(A\), the following equations can be written:

\[
A = \lambda N
\]

(5)

\[
\ln A = \ln A_0 - \lambda t
\]

(6)

or

\[
\log A = \log A_0 - \frac{\lambda}{2.3} t
\]

(7)

One common unit of activity is the curie (Ci).

\[1 \text{ curie (Ci)} = 3.7 \times 10^{10} \text{ dps or } 2.22 \times 10^{12} \text{ dpm}\]

Thus \(1 \text{ millicurie (mCi)} = 3.7 \times 10^{7} \text{ dps or } 2.22 \times 10^{9} \text{ dpm}\)

and \(1 \text{ microcurie (uCi)} = 3.7 \times 10^{4} \text{ dps or } 2.22 \times 10^{6} \text{ dpm}\)

Another unit, recommended to be used, is the Becquerel (one dps).

The parameter normally used to describe decay is the half-life \(T_{1/2}\) which is the time required for one half the number of radioactive atoms to disintegrate.

By definition, \(N = N_0/2\) for \(t = T_{1/2}\):

\[
\frac{1}{2} N_0 = N_0 e^{-\frac{\lambda}{2} T_{1/2}}
\]

(8)

Hence

\[
T_{1/2} = \ln 2/\lambda
\]

(9)

where

\[
T_{1/2} = \frac{0.693}{\lambda} \text{ in } t \text{ units}
\]

(10)

or

\[
\lambda = \frac{0.693}{T_{1/2}} \text{ in } t^{-1} \text{ units}
\]

(11)
It should be noted that \( \lambda \) and \( T_{1/2} \) are characteristic of a given radionuclide.

Substituting \( \lambda = \frac{0.693}{T_{1/2}} \) from equation (11) into equation (6) the following equation is obtained:

\[
\ln A = \ln A_0 - \frac{\ln 2}{T_{1/2}} t
\]

(12)

where \( A \) is the activity at time \( t \) and \( A_0 \) is the initial activity.

(Example).

If a sample containing \( ^{32}\text{P} \) has an activity of 10,000 counts per minute, what will be its activity after 30 days, knowing that for \( ^{32}\text{P} \) \( T_{1/2} = 14.3 \) days?

\[
\ln A = \ln 10,000 - \frac{\ln 2}{14.3} \times 30
\]

\[
\ln A = 9.21 - \frac{0.693}{14.3} \times 30
\]

\[
\ln A = 7.76
\]

\[
A = 2344 \text{ cpm}
\]

We could however estimate this activity without the need for log transformations with the following relationship:

after one half-life, \( \frac{A}{2} \) of the activity remains

after two half-lives, \( \frac{A}{2^2} \) of the activity remains

after \( n \) half-lives, \( \frac{A}{2^n} \) of the activity remains

Thus 30 days is approximately equal to 2 half-lives and the activity will be

\[
A = \frac{10000}{2^2} = 2500 \text{ cpm}
\]
8.2. Specific Activity

Activity, defined by equation (5), is frequently expressed in counts per minute or second (cpm, cps) or, if the counting efficiency is known, in disintegrations per unit time (dpm, dps). The activity depends on the size of the sample and so it is convenient to define a specific activity, to describe the ratio of the number of radioactive atoms to non-radioactive or carrier atoms of the same element, and even sometimes total weight or volume of the sample. As an example, specific activity of $^{32}\text{P}$ can thus be expressed as mCi $^{32}\text{P}/g^{31}\text{P}$.

8.3. 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 0.5 mCi $^{32}\text{P}/g\text{P}$ or 0.05 mCi $^{33}\text{P}/g\text{P}$ are frequently used in field studies.

In order to estimate the quantity of labelled fertilizer, 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). 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 $P_{dff} = 10\%$. Assume a counting efficiency of 40% (Cerenkov counting).
Agronomic specifications:

Phosphorus source: Ordinary superphosphate (OSP), 8.7 % P

P rate: 40 kg P/ha = 4 g P/m²

Plot size: 3.00 x 1.50 m = 4.5 m²

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 \( N \) will be 5000 dpm/2 g plant material.

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

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

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

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

\[
N_0 = \frac{16 \times 10^4 \text{ dpm}}{2.22 \times 10^9 \text{ dpm/mCi}}
\]

\[
= 7.2 \times 10^{-5} \text{ mCi} = 7.2 \times 10^{-2} \text{ uCi/2 g plant material}
\]

- Correction for chemical and biological dilution:

Amount of fertilizer - P

\[
= 2 \times \frac{0.25}{100} \times \frac{10}{100} = \frac{5}{10^4} \text{ or } 5 \times 10^{-4} \text{ g}
\]

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

\[
\frac{7.2 \times 10^{-2} \text{ uCi}}{5 \times 10^{-4} \text{ g P}} = 144 \text{ uCi } ^{32}P \text{ per g } ^{31}P
\]

This indicates that the \( ^{32}P \)-labelled fertilizer should have a minimum specific activity of 0.15 mCi/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 $\times$ \(4.5\,\text{m}^2/\text{plot} = 81\,\text{m}^2\)

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 would be 0.3 mCi/g P

The total activity for the experiment will therefore be:
\[324\,\text{g P} \times 0.3\,\text{mCi/g P} = 97\,\text{mCi}.\]

8.4. Choosing an appropriate isotope of phosphorus

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

In the previous example of a field study conducted for 70 days, whereas $^{32}\text{P}$ would decay through 5 half-lives to $1/2^5 = 1/32$ of its initial activity, $^{33}\text{P}$ 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 $^{33}\text{P}$. As $^{33}\text{P}$ is about 70 times more expensive than $^{32}\text{P}$, the economics would still favour $^{32}\text{P}$. 

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Given that $^{32}P$ has a half-life of 14.3 days and costs $0.50/mCi, while $^{33}P$ has a half-life of 25.3 days and costs $35/mCi, calculate the duration of a field study where the isotope cost would be equivalent (assuming equal counting efficiency).

Since the cost ratio is $35/0.5 = 70$ the initial activity of $^{32}P$ would have to be 70 times as high as $^{33}P$.

\[
\ln A = \ln A_0 - \frac{\ln 2}{T_{1/2}} t
\]

\[
\ln A = \ln 70 - \frac{\ln 2}{14.3} t = \ln 1 - \frac{\ln 2}{25.3} t
\]

\[
4.25 - 0.048 t = 0 - 0.027 t
\]

\[
t = \frac{4.25}{0.021} = 202 \text{ days}
\]

This exercise is thus only of academic interest, since a field study would not be conducted for 200 days. It clearly points out that $^{33}P$ labelled fertilizer would not be selected for a field experiment based on economics. It would however be difficult to obtain $^{32}P$ labelled fertilizer with a sufficiently high activity for an experiment lasting for more than 120 days (about 6 mCi $^{32}P/g$ P). If such material was available, high radiation safety precautions would be required and there would be concern that the radiation could affect plant growth. Under these circumstances $^{33}P$ labelled fertilizer would be the only alternative.
9. SUPPLIERS OF ISOTOPE LABELLED FERTILIZERS

The following are companies known to supply isotopically labelled fertilizers. The list is not complete, and many suppliers of isotopes exist for researchers who have the facilities to manufacture fertilizers from such products.

SUPPLIERS OF $^{15}$N LABELLED FERTILIZERS

PROCHEM
24 Deer Park Road
London SW19 3UF
England

ISO-COMMERZ GmbH
1115 Berlin-Buch
Lindenberger Weg 70
G.D.R.

MONSANTO Research Corporation
Mount Facility - Stable Isotope Sales
P.O. Box 32
Miamisburg, Ohio 45342
U.S.A.

Groupe CdF Chimie:Azote et Produits Chimiques
Place des Reflets,
Tour Aurore Cedex 5
92080 PARIS Defense (2)
France

SUPPLIERS OF $^{32}$P LABELLED FERTILIZERS

Tennessee Valley Authority
National Fertilizer Development Center (also $^{33}$P)
Muscle Shoals, Alabama 35660
U.S.A.