KNOWLEDGE DOCUMENT SERIES

M.A.L.A.Y.S.I.A.N N.U.C.L.E.A.R A.G.E.N.C.Y

ISOTOPIC TRACER TECHNIQUES FOR SOIL, NUTRIENT & WATER STUDIES

AUTHOR

Affrida Abu Hassan
Nur Humaira' Lau Abdullah
Anis Nadia Mohd Faisol Mahadeven
Ahmad Nazrul Abd Wahid
Mohd Noor Hidayat Adenan

NUKLEAR MALAYSIA

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ISOTOPIC TRACER TECHNIQUES FOR SOIL, NUTRIENT AND WATER STUDIES

1. Introduction

Isotopes are defined as atoms with the same proton number (atomic number) but different neutron number thus different atomic weight. The difference on the ratio of protons and neutrons influences the stability of the nucleus in an atom. Depending on the stability of the nucleus in an atom, isotopes may exist in stable and unstable forms. Unstable nucleus in an atom is called a radioactive isotope. In order for the unstable nucleus to gain stability, it spontaneously emits ionising radiation (alpha or beta particles and/or gamma electromagnetic rays). In contrast, a stable isotope is an atom with a stable nucleus hence does not spontaneously emit any radiation. Stable isotopes exist in light and heavy forms with heavy isotopes having a higher atomic weight than light isotopes.

Nuclear and isotopic techniques (also called nuclear-based techniques) are a complement to, not a substitute for, non-nuclear conventional techniques. The isotopic tracer technique (also known as atomic methods is labeled) was first proposed by GC de Hevesy and FA Paneth in 1913. This technique is based on the fact that the chemical properties of various isotope elements are very similar. In isotopic technique, the isotopes used can be either stable isotopes or radioactive isotopes (radioisotopes). Radioactive isotopes can be used to follow a particular element through various pathways and quantitative measurements may be made. They have the advantage of behaving in the same way that their stable counterparts do, but they can be readily traced. Stable isotopes are used in the same way as radioactive isotopes in soil/plant studies.

Isotopic techniques are useful and effective tools in agriculture where they are used to assess the soil water and nutrient status, particularly in the immediate vicinity of crop roots. These isotopes are used to trace soil water, evaluate water stress, water use efficiency, crop tolerance to drought and salinity, to evaluate soil fertility to identify hot spots of land degradation that deliver sediments and affect downstream water quality, to quantify N_2 fixation by legume crops and N-fixing bacteria as well as to evaluate uptake efficiency and utilization of fertilizer input (w.r.t nitrogen, phosphorus and potassium) under different water conditions of industrial and food crops. Such information assists in developing strategies for sustainable agricultural water management. To date, more than 50 related to soil water management projects have been conducted in Malaysian Nuclear Agency, including research and development, technical and advisory services, laboratory support, training in the use of nuclear techniques and assistance in collecting, analyzing and disseminating the derived information and technology.

a. Incredible Isotopes

b. What Are Radioactive Isotopes?

c. Building Better Agriculture One Atom at a Time

d. More Crop Per Drop

e. Using Nuclear Science to Manage Nitrogen

In soil, water and nutrient management projects, most of the previous research works were conducted to evaluate uptake efficiency and utilization of fertilizer input (w.r.t nitrogen, phosphorus and potassium) under different water condition of industrial and food crops such as rubber, oil palm, pineapple, rice, groundnut, banana, cocoa plantation and others. Two main nuclear techniques were used, viz isotopic technique (^{15}N, ^{32}P, ^{86}Rb, etc.) as tracer and soil moisture neutron probe (SMNP) for soil moisture measurement. Results from the research works provided recommendation on water application strategy for sufficient crop water management subject to meteorological prediction.

Recently, isotopic technique using carbon isotope was introduced for agricultural research in Nuklear Malaysia. Carbon is an important building component of plants. Green plants assimilate carbon from atmospheric carbon dioxide (CO_2) through the process of photosynthesis. Carbon dioxide is composed of two stable isotopes, the less abundant ^{13}C and the lighter ^{12}C. During photosynthesis the plant discriminates against the heavier isotope in favor of the lighter one. The extent of this discrimination depends on environmental factors, such as water availability and salts in the soil. The variation in the relative abundance of the carbon isotopes can therefore be used as a surrogate marker of water stress, water use efficiency and crop tolerance to drought and salinity.

The ^{15}N isotope dilution technique is used to quantify N_2 fixation by legume crops and N-fixing bacteria. The advantage of the ^{15}N isotope dilution method is that it makes it possible to separate N taken up by the plant from fertilizer and soil from that fixed in the plant. Many researchers have described the ^{15}N isotope dilution method as the most reliable measure of N_2 fixation. A variety of crops and area were used in the study. If in the 1980s the study only focused on leguminous crops, at present the study has focused on evaluation of biofertilizer products, containing N_2 fixing microorganisms, on crops such as rice, oil palm, vegetables, vanilla orchids and groundnut. Nuklear Malaysia has been giving consultation on BNF for its ^{15}N isotopic analysis services and expertise on the research.

1. Soil and Water Management through Isotopic Techniques and Precision Agriculture
2. Role of Isotopes in Soil-Plant, Water and Fertility Research

2. Research Activities on Soil, Water & Nutrient Study Using Isotopic Tracer Techniques

Currently, the studies that involving the use of the isotope tracer technique are focused on nitrogen use efficiency and water use efficiency of industrial crops such as rice and kenaf. It is because, the nitrogen fertilizer and water issue has become a major problem to the agricultural industries in Malaysia due to the increasing of nitrogen fertilizer utilization, prizes, exceeding and wasting use of nitrogen fertilizer and climate change. It also involves studies on the N_2 fixation as an alternative nitrogen supply to plants through useful microbes in biofertilizer products develop by Nuklear Malaysia.

3. Nitrogen use Efficiency Study on Crops

Among the nitrogen use studies conducted were under the science fund project "*Nutrient Dynamic on Interaction between Nitrogen and Carbon Nutrition and Water Management for Improving of Aerobic Rice Productivity using Isotope Discrimination Technique*" (06-03-01-SF0203) and IAEA/RCA RAS5073 project "*Supporting Climate-Proofing Rice Production Systems (CRiPS) Based on Nuclear Application"*. These studies aimed to improve the efficiency of nitrogen fertilizer utilization on rice crops under flooded and aerobic condition which may have an impact on the reduction of nitrogen fertilizer consumption by the country thus reducing wastage of nitrogen fertilizer. Several experiments were conducted in this study involving the use of ^{15}N isotope tracer techniques at the field and glass house. The study also involved the cooperation between Nuklear Malaysia and the Integrated Agriculture Development Authority (IADA) where all field experiments were conducted at a paddy field in Sepayang in Rompin, Pahang. Rice mutant lines NMR151 and NMR152 were also used as study crops.

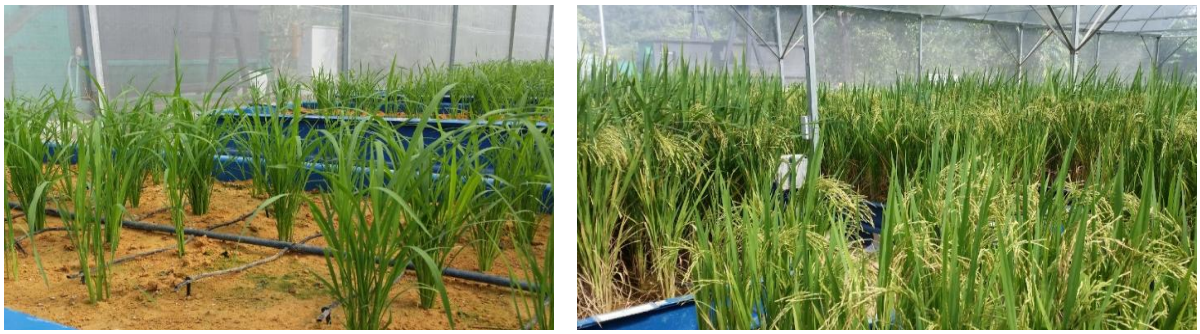

Figure 1: NUE study on rice mutant line in shade house.

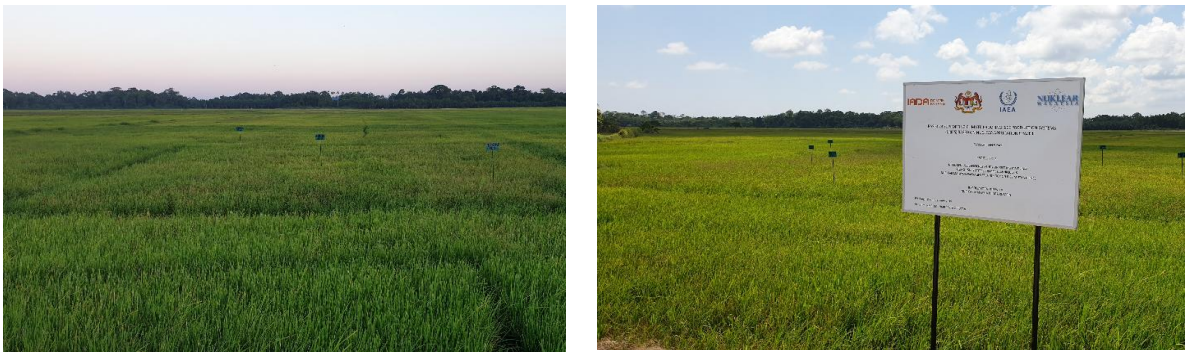

Figure 2: NUE study on rice mutant line at Sepayang in Rompin, Pahang

Another crop studied was kenaf, under the project IAEA/RCA RAS5070 *"Developing Bioenergy Crops to Optimize Marginal Land Productivity through Mutation Breeding and Related Techniques".* This study aimed to improve the efficiency of nitrogen fertilizer use on kenaf crops under normal and marginal land. It also aimed to improve the agronomic management of kenaf mutant line crops on marginal land. Several experiments were conducted in this study involving the use of ^{15}N isotope tracer techniques at the field and glass house. The study also involved the cooperation between Nuklear Malaysia and National Kenaf and Tobacco Board (LKTN). The glass house experiments were conducted in Nuklear Malaysia while the field experiments were conducted at LKTN Beseri, Perlis and LKTN Setiu, Terengganu. Kenaf mutant lines produced by Nuklear Malaysia were used as the study crop. The impacts of this study are better fertilizer management, reduction of cost and the utilization of marginal land as the kenaf planting area by the country.

Figure 3: NUE study on kenaf mutant line on marginal land at LKTN Setiu.

4. Water Management Study on Rice Crops

This study was conducted under the science fund project "*Nutrient Dynamic on Interaction between Nitrogen and Carbon Nutrition and Water Management for Improving of Aerobic Rice Productivity using Isotope Discrimination Technique*" (06-03-01-SF0203). This study aimed to improve water use efficiency (WUE) and water management of rice mutant lines NMR151 and NMR152 which could potentially save water and better water manage rice crops. It also aimed to introduce the drought tolerant rice cultivar produced by Nuklear Malaysia. Several experiments were conducted in this study involving the use of ^{13}C discrimination technique in the field and greenhouse. The use of observations from ^{13}C isotope discrimination provided an alternative tool to evaluate the impact of water stress on crop performance and to estimate water use efficiency.

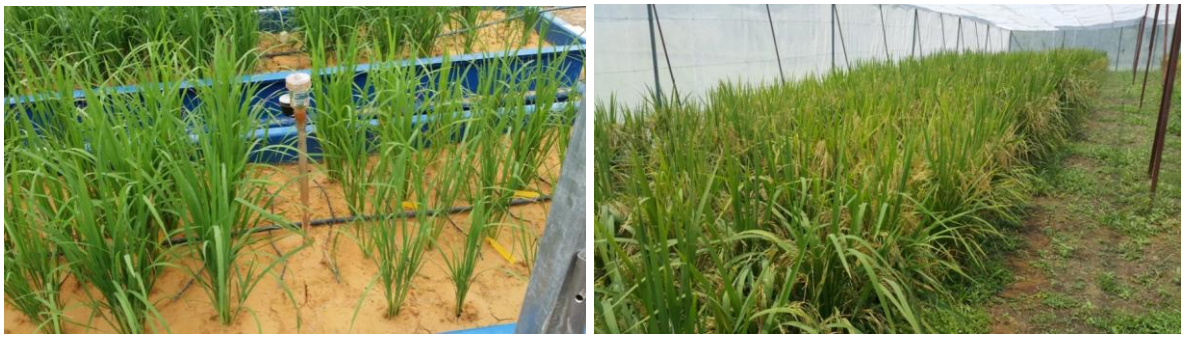

Figure 4: Water use efficiency study on rice mutant line in shade house.

5. ^{13}C Isotope Discrimination Study on Crops

This study was conducted under project IAEA/RCA RAS5070 *"Developing Bioenergy Crops to Optimize Marginal Land Productivity through Mutation Breeding and Related Techniques".* This study aimed to improve water use efficiency and water management of kenaf mutant lines. In this study, the experiment was conducted only in green house. The study involved the use of ^{13}C discrimination technique as a tool to evaluate the impact of water stress on crop performance and water use efficiency. The impacts from this study were water saving and better water management on kenaf crops. It also enabled evaluation of growth performance of the kenaf mutant lines under water stress condition.

Figure 5: Water use efficiency study on kenaf mutant lines in shade house.

6. ^{15}N isotopic dilution technique in biofertilizer study

Biofertilizer is a substance which contains living microorganisms, which promotes plant growth by increasing the supply or availability of primary nutrients to the host plants. Nuklear Malaysia has carried out biofertilizer development project under the initiatives of Forum for Nuclear Cooperation in Asia (FNCA) Biofertilizer Project. This initiative aims to support sustainable agriculture in Asia as the use of environmental friendly biofertilizer can reduce the usage of the chemical fertilizer. Incorporating nuclear technologies in biofertilizer development to the industry comes with its challenges and potentials. In biofertilizer study, stable isotope of nitrogen, nitrogen-15 can be effectively utilized as a tracer to accurately quantify the efficiency of biofertilizer microorganisms. The technique is highlighted in Biofertilizer Manual, FNCA. Studies have been conducted in order to determine the contribution of several formulations of Nuklear Malaysia biofertilizer products in supplying plant with nitrogen using ^{15}N isotopic dilution technique. Various biofertilizer products and plants have been studied either in field or greenhouse trials as described in Table 1.

Table 1: Field and glass house trials of biofertilizer products.

	**Plant:** Kenaf mutant line **Category:** Field trial **Location:** Beseri, Perlis **Biofertilizer:** Bioliquifert **Reference:** 1. Biofertilizer study on kenaf mutant lines using isotopic tracer technique at Beseri Perlis. 2. Standard Operating Procedure Application of ^{15}N Isotopic Tracer in Nitrogen and Biofertilizer Study on Kenaf Crop
	**Plant:** Maize **Category:** Field trial **Location:** Sungkai, Perak **Biofertilizer:** M99
	**Plant:** Chinese cabbage **Category:** Glass house trial **Biofertilizer:** AP1, AP2, AP3 **Reference:** 1. Development of multifunctional biofertilizer formulation from indigenous microorganisms and evaluation on their N2-fixing capabilities on Chinese Cabbage using 15N tracer technique 2. Multifunctional biofertilizer products for sustainable agriculture

	**Plant:** Okra **Category:** Glass house trial **Biofertilizer:** AP2, AP3 **Reference:** Development of seed treatments biofertilizer and nutrient uptake study using tracer technique for okra plants
	**Plant:** Paddy **Category:** Glass house trial **Biofertilizer:** Bioliquifert **Reference:** Assessment of multifunctional biofertilizer on rice in greenhouse trial

7. Findings Summary Of Study Using Isotopic Tracer Techniques

Through the studies involving the use of tracing isotope techniques in agriculture, many useful data that give benefits to the country are available. In plant nutrition study, researchers have found that nitrogen fertilizer application rate given to plants in some area are very high. For example, the normal farmers practice of application nitrogen fertilizers is 120 kg N/ha. However, the study showed some area only requires nitrogen fertilizer rate at 80 kg N/ha to obtain better revenue to the farmers. The difference between nitrogen fertilizers application by farmer practices with the recommended rates through the study will actually give more benefits to the country in terms of saving on nitrogen fertilizer usage. The reduction of nitrogen fertilizer application usage will decrease the farmers input cost and also give a better impact to the environment. It leads to reduction in import of nitrogen fertilizers by the country.

The study also found that the use of biofertilizers (nitrogen fixation) could reduce almost half the use of nitrogen fertilizers. Biofertilizer produced by Nuklear Malaysia could be an alternative to the use of chemical fertilizers in which it saves nitrogen fertilizer usage. For rice crops, the study found that the nitrogen use efficiency by the traditional method (flood method) was less than 40% which showed that the nitrogen fertilizer provided was not fully utilized by the crops. However, through the study, aerobic condition methods of rice planting (non-flood method) were found to increase the nitrogen use efficiency to more than 40%. It also improves water use efficiency where almost 40% of water savings occurred.

8. Benefits Of Using Isotopic Tracer Technique In Agroecosystem Study

The use of isotopic tracer techniques in agroecosystem study gives many advantages and benefits to nutrient management, soil and water of crops. The use of ^{15}N isotope technique can potentially pave way for a better nitrogen fertilization management. It is known that the import and consumption of nitrogen fertilizers in Malaysia is high. Malaysia has spent millions to import nitrogen fertilizers. The high and uncontrolled usage of nitrogen fertilizers also causes losses and leads to soil and environmental pollution (explain details). Through fertilization studies involving the use of ^{15}N tracer isotope technique, a better recommendation on nitrogen fertilizer application can be decided by the country in terms of reducing the agricultural input cost and save the environmental from the excessive usage of nitrogen fertilizer usage contamination.

Using isotopic tracer technique also gives benefits to the water utilization in agroecosystem. G, (explain) climate change causes water scarcity to some place in the country. The use of ^{13}C isotope discrimination is the alternative way to study water use efficiency in plant and water relationship. Using this technique, we can improve the cultivation and production of Nuklear Malaysia new crop varieties showing tolerance to drought or water stress. Better water management will lead to water savings by the country.

1. Standard Operating Procedure of NOI7
2. Video on Analysis of 15N using NOI7 Emission Spectrometer

9. NOI7 Emission Spectrometer

The NOI7 (Figure 6) allows the determination of ^{15}N – contents in relation to ^{14}N – contents. The measuring range covers concentrations from the natural abundance (0.3663%) up to enrichments of 80% ^{15}N. Thus the NOI7 excellently meets demands of the determination of the relative ^{15}N – abundance in sample originating from ^{15}N – tracer experiments. Hence, the NOI 7 finds its applications within such fields like biology and medicine where problems are pondered relevant to nitrogen. Especially, these are studies on fertilizer efficiency, questions within the field of nitrogen fixation in ecosystems and investigations in the protein metabolism as well as gastroenterological examinations.

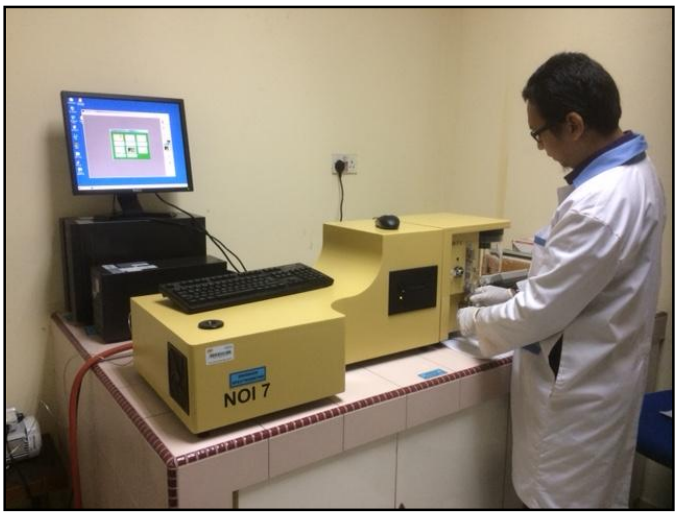

Figure 6: NOI7 Emission spectrometer

In comparison with experiments using radioactive isotopes the use of stable isotope ^{15}N is particularly advantageous, because a pollution by radioactive materials is completely avoided. Therefore, no radiation protection regulations whatever have to be observed and no objections need to be made to the application of ^{15}N tracer technique to man or to human or animal food.

9.1 Consumables in NOI7

There are three main consumables required for the analysis of ^{15}N atom percent using NOI7 viz.; sodium hypobromite solution (Figure 7(a)), sample glass (Figure 7(b)) and pipette tips.

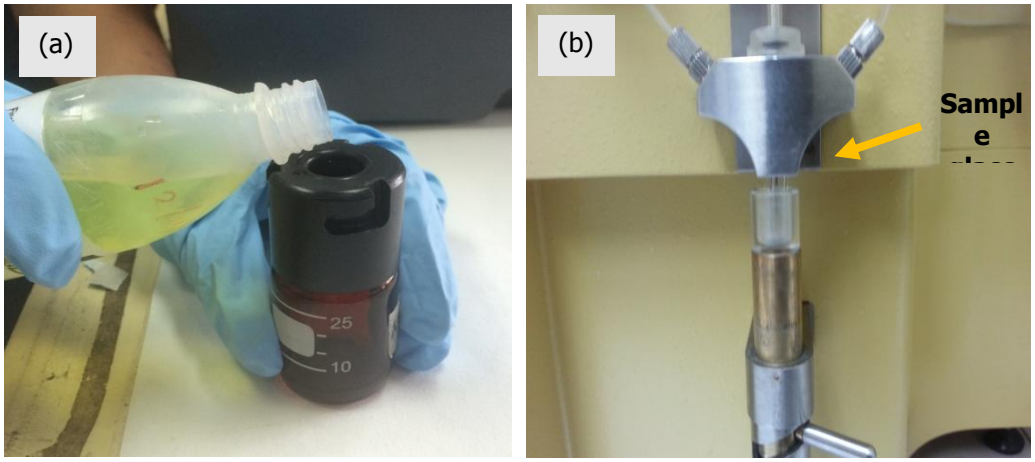

Figure 7: (a) Sodium hypobromite solution and (b) sample glass.

9.2 The Operating Principle of the NOI7

In nitrogen gaseous (N_2) is carried by a continuous Helium carrier gas flow into NOI7. Due to the excitation within an electrical HF field at a reduced pressure the nitrogen emits an UV radiation. In a grating polychromator, the emitted light, which contains the information about the three possible combinations (Table 2) of the two stable isotopes ^{14}N and ^{15}N, is split into its spectral components and converted into an electrical signal which will be digitally processed.

Table 2: Three possible combinations of the two stable isotopes 14N and 15N

Molecule	$^{14}N_2$	$^{14}N\ ^{15}N$	$^{15}N_2$
Wavelength	297.68 nm	298.29 nm	298.86 nm

There is a chemical sample preparation system in NOI7 in order to generate the N_2 from liquid samples (aqueous solutions of ammonium chloride, ammonium sulphate, and urea – “chemical samples”). Recommended liquid sample types is a product from Kjeldahl method which went through digestion, distillation and titration methods. Within the NOI7 the molecular nitrogen is released from the ammonium-containing sample solution by addition of an aqueous sodium hypobromite solution (NaOBr) and carried to the basic unit by a continuous Helium stream. High-precision components ensure the constant Helium stream and pressure needed in this process. All supplementary safety options from the previous prototype are now integrated as standard in the NOI7.

Samples that are different from those mentioned above can be digested using a DUMAS combustion (e.g. Kjeldahl method) unit which is incorporated in an elementary (combustion samples). Hence it is necessary to equip the NOI7 for the detection of the external start pulse and, if existing, for the NOI7 carrier gas connection.

9.3 Calibration

Two types of calibration are required for each measurement method in order to get exact and reproducible results. They are:

a. Isotope calibration

The first available result at the end of each measurement is a machine value provided by the analyzer, thus an isotope calibration for the automatic conversion into the corresponding ^{15}N abundance is required. The calibration curve of the NOI7 runs sufficiently linear, therefore the calibration graph can be produced by the program routine by interpolation of the calibration values adding an extrapolation of 20% to the last calibration values.

Because of the spectral interference of the Nitrogen peaks the calculation method has to be altered, starting at a ^{15}N abundance of 33% ^{15}N (raw value). As this is accompanied by a shift of the machine values, a second calibration series id required for the high abundance range. A basic calibration is provided by the producer. A new calibration is required if during the control measurement with standard samples an unacceptable difference of the calculated abundance of isotopes to the real value occurs. As it is not possible – for physical reason - to produce a valid calibration graph for a certain kind of samples by using another kind of samples, to each method a respective calibration must be allocated.

b. Background correction

As in line with the content of Nitrogen in the Helium carrier gas also the excitement conditions in the discharge tube change, the side bands of Nitrogen not used for the measurement are differently strongly excited for light emission in relation to the Nitrogen content. As furthermore these emissions interfere with the range of the Nitrogen spectrum most expedient for the ^{15}N detection, the optical background of the measurement spectrum will also be influenced by the Nitrogen content. Because for the examination of the $^{14}N/^{15}N$ ratio the optical background must be subtracted from the determined peak heights and thus a correction of the influence mentioned above is indicated for big differences in the content of Nitrogen in the samples. The amount of influence in the result can be examined by measuring standard samples with natural ^{15}N abundance with Nitrogen contents that cover the entire work range. It can be sufficiently exactly described with a factor of intensity of the measurement spectrum.

The background correction is already provided by the producer and is in general only required after replacement of optical bits or for new kind of samples. A background calibration is required for several standard samples with a constant abundance of isotopes but varying content of Nitrogen a tendency of the isotope value can be detected. This has to be considered especially concerning small contents of Nitrogen. A calibration series for the background correction is only valid for the same method of measurement gas production and has to be recorded separately for each particular kind of sample. That also includes separate calibrations for samples with considerably varying descent.

RUJUKAN

Publications:

1. Zaman, M. et al. The Role of Nuclear Techniques in Biosaline Agriculture. Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques (2018). doi:10.1007/978-3-319-96190-3_6 (https://link.springer.com/chapter/10.1007%2F978-3-319-96190-3_6#copyrightInformation)

2. Fallis, A. Use of isotope and radiation methods in soil and water management and crop nutrition. J. Chem. Inf. Model. (2013). doi:10.1017/CBO9781107415324.004 (http://www-naweb.iaea.org/nafa/swmn/public/tcs-14.pdf)

3. Singh, B., Singh, J. & Kaur, A. Applications of Radioisotopes in Agriculture. Int. J. Biotechnol. Bioeng. Res. 4, 167–174 (2013). (http://www.ripublication.com/ijbbr_spl/ijbbrv4n3spl_02.pdf)

4. Dileep, K. & Singh, A. P. Role of Radioisotopes in soil plant system. 4, 3348–3351 (2013). (https://www.researchgate.net/publication/294087094_Role_of_Radioisotopes_in_soil_plant_System)

5. Zapata, F. et al. Innovations in Soil and Water Management/Conservation Research through Integrated Approaches of Nuclear and Isotopic Techniques and Precision Agriculture. 247–282 (2015). doi:10.1201/b18759-11

   (https://www.researchgate.net/publication/283005437_Innovations_in_Soil_and_Water_ManagementConservation_Research_through_Integrated_Approaches_of_Nuclear_and_Isotopic_Techniques_and_Precision_Agriculture)

6. Nazrul, A. et al. Nitrogen Use Efficiency and Carbon Isotope Discrimination Study on NMR151 and NMR152 Mutant Lines Rice at Field under Different Nitrogen Rates and Water Potentials. (https://inis.iaea.org/collection/NCLCollectionStore/_Public/48/050/48050401.pdf?r=1&r=1)

7. Approaches to Improvement of Crop Genotypes with High Water and Nutrient Use Efficiency for Water Scarce Environments IAEA TECDOC SERIES. In (https://www.iaea.org/publications/12287/approaches-to-improvement-of-crop-genotypes-with-high-water-and-nutrient-use-efficiency-for-water-scarce-environments)

8. Role of Isotopes in Soil-Plant, Water and Fertility Research. VI, (2007).

(https://www.researchgate.net/publication/309322433_Role_of_Isotopes_in_Soil-Plant_Water_and_Fertility_Research)

Video:

a. Incredible Isotopes (https://youtu.be/R2XWlhNz6WU)

b. What Are Radioactive Isotopes? (https://youtu.be/UYvx0O8itMA)

c. Building Better Agriculture One Atom at a Time (https://youtu.be/LiR2RG9uCMI)

d. More Crop Per Drop (https://youtu.be/vbaTz8TO55E)

e. Using Nuclear Science to Manage Nitrogen (https://youtu.be/wC2f8hMd3-

f. Video of Applicationn of 15N Tracer (https://sp-kms.nuclearmalaysia.gov.my/ppt/bab/Video%20Penyelidikan/15-N%20Isotope%20Application?Web=1)