IAEA TECDOC SERIES

IAEA-TECDOC-1870

Sample Preparation of Soil and Plant Material for Isotope Ratio Mass Spectrometry

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

IAEA
International Atomic Energy Agency

SAMPLE PREPARATION OF SOIL AND PLANT MATERIAL FOR ISOTOPE RATIO MASS SPECTROMETRY

The Agency's Statute was approved on 23 October 1956 by the Conference on the Statute of the IAEA held at United Nations Headquarters, New York; it entered into force on 29 July 1957. The Headquarters of the Agency are situated in Vienna. Its principal objective is "to accelerate and enlarge the contribution of atomic energy to peace, health and prosperity throughout the world".

IAEA-TECDOC-1870

SAMPLE PREPARATION OF SOIL AND PLANT MATERIAL FOR ISOTOPE RATIO MASS SPECTROMETRY

PREPARED BY THE
JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 2019

COPYRIGHT NOTICE

For further information on this publication, please contact:

Soil and Water Management and Crop Nutrition Section
International Atomic Energy Agency
Vienna International Centre
PO Box 100
1400 Vienna, Austria
Email: Official.Mail@iaea.org

Printed by the IAEA in Austria
May 2019

IAEA Library Cataloguing in Publication Data

Names: International Atomic Energy Agency.
Title: Sample preparation of soil and plant material for isotope ratio mass spectrometry / International Atomic Energy Agency.
Description: Vienna : International Atomic Energy Agency, 2019. | Series: IAEA TECDOC series, ISSN 1011–4289 ; no. 1870 | Includes bibliographical references.
Identifiers: IAEAL 19-01234 | ISBN 978–92–0–102019–2 (paperback : alk. paper)
Subjects: LCSH: Mass spectrometry. | Sample preparation (Chemistry). | Stable isotopes.

FOREWORD

Carbon and nitrogen are the main nutrients for plants and play a major role in biological processes. Carbon and nitrogen isotopic tracer techniques can be used to better understand nutrient dynamics, as well as carbon and nitrogen cycles. Fertilizers that have been enriched with ^{15}N can be used to study fertilizer use efficiency and biological nitrogen fixation, while ^{13}C isotopes provide information on drought and salinity stress.

Appropriate sample preparation is the first step to ensure the quality of stable isotope analysis. Information on sample preparation for such analysis is often described in a summarized, non-comprehensive way, without proper illustration of each of the steps involved. This is a major constraint for scientists in developing countries. Often, sample volumes of harvested soil or plant material need to be reduced prior to grinding, cross-contamination must be avoided, and the final sample must be representative and in adequate concentration for isotope ratio mass spectrometry.

This publication focuses on sample preparation. The standard operating procedures presented here provide comprehensive instructions in quartering/sub-sampling, grinding and weighing samples for isotope ratio mass spectrometry to determine the $\delta^{15}N$ and $\delta^{13}C$ composition of plant and soil material.

The present publication is expected to be a valuable resource for technicians and fellowship trainees, and provides useful information for scientists and students.

The Joint FAO/IAEA Division wishes to thank all the contributors involved in the preparation of this publication. The IAEA officers responsible for this publication were G. Dercon, L. Heng, M. Heiling and C. Resch of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

EDITORIAL NOTE

This publication has been prepared from the original material as submitted by the contributors and has not been edited by the editorial staff of the IAEA. The views expressed remain the responsibility of the contributors and do not necessarily represent the views of the IAEA or its Member States.

Neither the IAEA nor its Member States assume any responsibility for consequences which may arise from the use of this publication. This publication does not address questions of responsibility, legal or otherwise, for acts or omissions on the part of any person.

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

The mention of names of specific companies or products (whether or not indicated as registered) does not imply any intention to infringe proprietary rights, nor should it be construed as an endorsement or recommendation on the part of the IAEA.

The IAEA has no responsibility for the persistence or accuracy of URLs for external or third party Internet web sites referred to in this publication and does not guarantee that any content on such web sites is, or will remain, accurate or appropriate.

CONTENTS

1. INTRODUCTION 1

1.1. BACKGROUND 1
1.2. OBJECTIVE 1
1.3. STRUCTURE 1

2. SAMPLE CHOPPING AND QUARTERING OF PLANT MATERIAL FOR ISOTOPE RATIO MASS SPECTROMETRY 3

2.1. SCOPE AND FIELD OF APPLICATION 3
2.2. BACKGROUND INFORMATION AND CONSIDERATIONS 3
2.3. EQUIPMENT 3
2.4. PROCEDURE 3

3. SAMPLE MILLING FOR ISOTOPE RATIO MASS SPECTROMETRY 6

3.1. SCOPE AND FIELD OF APPLICATION 6
3.2. BACKGROUND INFORMATION AND CONSIDERATIONS 6
3.3. EQUIPMENT 7
3.4. PROCEDURE 7
3.4.1. Rotor mill 7
3.4.2. Mortar grinder 9
3.4.3. Ball mill 11
3.4.4. Ball mill with adapter for microcentrifuge tubes 13

4. SAMPLING PLANT MATERIAL THROUGH PUNCHING 15

4.1. SCOPE AND FIELD OF APPLICATION 15
4.2. BACKGROUND INFORMATION AND CONSIDERATIONS 15
4.3. EQUIPMENT 15
4.4. PROCEDURE 16

5. SAMPLE WEIGHING FOR ISOTOPE RATIO MASS SPECTROMETRY 18

5.1. SCOPE AND FIELD OF APPLICATION 18
5.2. BACKGROUND INFORMATION AND CONSIDERATIONS 18
5.3. REAGENTS 18
5.4. EQUIPMENT 19
5.5. PROCEDURE 19
5.5.1. Weighing of solid samples 19
5.5.2. Encapsulation of liquid samples 22
5.5.3. Optional: recording of sample weights using an Excel macro 23

6. CARBONATE REMOVAL FROM SOIL FOR DETERMINATION OF ORGANIC CARBON BY ISOTOPE RATIO MASS SPECTROMETRY 27

6.1. SCOPE AND FIELD OF APPLICATION 27
6.2. BACKGROUND INFORMATION AND CONSIDERATIONS 27
6.3. REAGENTS 27
6.4. EQUIPMENT 28

6.5. PROCEDURE 28
6.5.1. Acid fumigation of soils to remove carbonates 28
6.5.2. Direct acidification of soils to remove carbonates 31

1. INTRODUCTION

1.1. BACKGROUND

Carbon (C) and Nitrogen (N) are major plant macronutrients and central to biogeochemical cycles. Their stable isotopes form the backbone of a vast array of tracer methods for understanding C and N cycling in agricultural and ecological systems. Examples include the quantification of biological nitrogen fixation, monitoring N fertilizer use efficiency, assessing plant responses to drought stress and determining the magnitude of C sequestration. Isotope Ratio Mass Spectrometry (IRMS) analysis is by far the most common analytical method in these approaches.

Over the last decades, IRMS has become affordable, resulting in widespread application of δ15N and δ13C isotopic techniques in agricultural sciences. While the accuracy and precision of IRMS are high for C and N if the material is uniform (±0.2‰), IRMS requires very small amounts of sample. Therefore, the quality of the results hinges on the homogeneity and representativeness of this sample. Extensive experience of the Soil and Water Management & Crop Nutrition Laboratory has highlighted that standardized sample preparation is crucial to success and is often lacking.

1.2. OBJECTIVE

This TECDOC aims to equip laboratories and practitioners with detailed guidelines on how to obtain homogeneous and representative samples of soil and plant material for IRMS analysis of stable isotopes of C and N, thus ensuring high quality and comparable analytical results. Special attention is given to the need for avoiding cross-contamination when working with both natural abundance and enriched material.

1.3. STRUCTURE

Five Standard Operating Procedures (SOP's) guide the reader on how to obtain representative and homogeneous sub-samples for isotope analyses. Specifically, step-by-step instructions describe (1) quartering of plant materials, (2) grinding of plant and soil samples, (3) sampling through punching of plant tissue, (4) inorganic carbonate removal, and (5) weighing of samples into tin cups for IRMS analysis.

The first SOP covers the representative volume/weight reduction of harvested plant material to ensure identical isotopic composition of the sub-sample and the original harvested material.

The second SOP compares different methods for grinding plant and soil material to the required fineness. Having sampling material with a homogenous particle size and fineness is essential to be able to obtain representative sub-samples of very small volumes.

The third SOP explains how to use a marginally destructive method (punching of plant tissue) to sample plant material. This method can be applied when the same plants must be analyzed at various growth stages.

The fourth SOP focuses on preparing the tin cups for IRMS analysis, in order to prevent sample leakage from the tin cup resulting in cross-contamination during the measurement. This section of the TEDOC can be applied for isotope analysis of soils and plants as well as food, insects or any other solid material, containing C and N.

The fifth SOP describes a method to remove inorganic carbonates of soil samples. This soil pre-treatment is necessary if organic and inorganic C must be distinguished, e.g. if organic matter is the aim of the research.

2. SAMPLE CHOPPING AND QUARTERING OF PLANT MATERIAL FOR ISOTOPE RATIO MASS SPECTROMETRY

2.1. SCOPE AND FIELD OF APPLICATION

This SOP covers the chopping and quartering of plant samples.

2.2. BACKGROUND INFORMATION AND CONSIDERATIONS

Field experiments produce yields in the range of kilograms fresh weight of plant material per plot. For IRMS analysis, only a few milligrams of dry plant material are analyzed per sample, so obtaining a small, representative and homogenous sample out of bulk sample amounts is an essential step of sample preparation.

Special care should be taken when working with ^{15}N or ^{13}C enriched plant material. Best practice is to chop natural abundance and enriched samples separately. In case this is not achievable due to limitations of instruments, follow these steps:

- Never chop natural abundance and enriched samples at the same time.
- After chopping of enriched samples, clean the chopper with compressed air and a water hose. Chop minimum 10 kg natural abundance dummy plant material and discard this material. Clean the chopper with compressed air and a water hose to completely remove traces of enriched samples.
- If possible chop natural abundance material first.

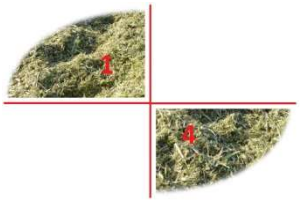
2.3. EQUIPMENT

- Garden chipper/shredder (corded or preferably equipped with a petrol engine for operation independently of the electricity network, minimum branch diameter: 40 mm)
- Secateurs

2.4. PROCEDURE

Step	Procedure	
Step 1 Cutting by hand	Small sample amounts can be cut by hand with secateurs.	

Chopping	Bulk amounts resulting from field experiments should be chopped with a chipper.	
Step 2 Quartering	Put the chopped plant sample on a big clean table and form a pile. Mix the sample thoroughly with hands.	
	In case you must do the quartering in the field, put the chopped plant sample on a canvas. Mix the sample thoroughly with hands or a hay fork.	
	Divide the pile into 4 quarters.	
	Discard quarter 2 and quarter 3.	

	With the remaining chopped material, form a pile and mix the sample thoroughly with hands.	
	Divide the smaller pile into 4 quarters again.	
	Discard quarter 2 and quarter 3.	
	Repeat the mixing and quartering steps until you end up with a few hundred grams of fresh plant sample.	
Step 3 Drying	Dry the plant samples in the oven at 65°C. Generally it takes 3 days, until sample weight is constant. After drying the samples are ready to be milled.	 

3. SAMPLE MILLING FOR ISOTOPE RATIO MASS SPECTROMETRY

3.1. SCOPE AND FIELD OF APPLICATION

This SOP covers the milling of solid soil and plant samples. This procedure is essential prior to IRMS analysis. The proposed procedure is relevant to any model of mills of any provider.

3.2. BACKGROUND INFORMATION AND CONSIDERATIONS

Plant samples must be chopped or cut and dried at 65°C prior to milling. Soil samples are sieved to 2 mm and dried at 40°C.

Completely dry material is easier to cut. Therefore, samples for milling should be taken directly from the warm oven to prohibit water absorption.

Depending on the sample size and material, different types of mills are available for IRMS sample preparation: Ball mills can be used for plant and soil samples and will produce excellent results in terms of fineness of the samples. On the other hand, ball milling is quite time consuming and only suitable for smaller sample amounts. Rotor mills will speed up the milling process of plant samples and are able to process larger amounts of material but will produce coarser end products. Mortar mills are perfect for processing bulk amounts of soil samples within short time. The advantages and disadvantages of different mills are listed in Table 2.1.

TABLE 2.1. LIST OF PROPERTIES OF DIFFERENT MILLS

Type of mill	Advantage	Disadvantage	Most suitable for
Rotor mill	- fast - for large amounts of sample	- coarser end products - cleaning takes more time	soil and plant
Mortar grinder	- even for hard components such as small stones - fast and easy cleaning	- not suitable for plant fibres - milling is time consuming	soil
Ball mill	- excellent results in fineness of samples - fast and easy cleaning	- milling is time consuming	soil and plant
Ball mill with adapter for microcentrifuge	- up to 48 samples can be ground in one step - very small amounts of samples can be ground without any losses	- only suitable for nonabrasive samples such as plants - possibility of contamination resulting from plastic vials (only relevant for $\delta^{13}C$ analysis)	plant

Special care must be taken with ^{15}N enriched samples. Best practice is to mill natural abundance and ^{15}N enriched samples in separate rooms with separate mills. In case this is not achievable due to limitations of instruments and space, follow these steps:

- Never mill natural abundance and enriched samples at the same time.
- After milling of enriched samples clean the mill with compressed air. Mill minimum 10 natural abundance dummy samples and clean the mill after each dummy sample with compressed air to completely remove traces of enriched samples.
- Dispose of the dummy samples.
- Clean the workspace thoroughly with alcohol and all tools with compressed air and alcohol.

3.3. EQUIPMENT

- Mill
- Steel balls
- Microcentrifuge tubes 1.5 mL

3.4. PROCEDURE

3.4.1. Rotor mill

Step	Procedure	
Step 1 Switch on	Switch on the mill.	
Step 2 Preparation	Check if all grinding tools are clean, if not, clean with compressed air.	

	Insert the collecting vessel and the ring sieve, close the housing cover.	
Step 3 Set parameters of the mill	Set the speed. 15000 rpm is a good starting point.	
Step 4 Milling	Set the time to infinite and press the start button.	
	Feed the sample in portions from above. Note: For samples with a particle size of more than 2cm, an additional grinding may be necessary. For such additional step, a mill for coarse material can be used.	
	Press the on/off button to end the milling manually.	

	Open the housing cover, remove the collecting vessel and the ring sieve and take out the ground sample from the collecting vessel. Clean the milling tools with compressed air.	

3.4.2. Mortar grinder

Step	Procedure	
Step 1 Switch on	Switch on the mill.	
Step 2 Preparation	Check if the grinding tools are clean, if not, clean with compressed air. Ensure that the mortar is fitted correctly in the groove at the bottom plate.	
Step 3 Set parameters of the mill	Set the time. Usually 3 minutes are sufficient.	

Step 4 Milling	Close the housing cover and press the start button.	
	Feed the sample into the mortar.	
	Either wait until the countdown has expired or press the stop button to end the milling manually.	
	Open the housing cover and take out the mortar.	
	Take out the ground sample from the mortar. Clean the milling tools with compressed air.	

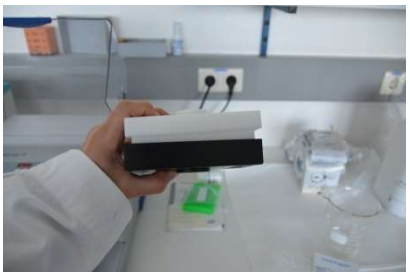
3.4.3. Ball mill

Step	Procedure	
Step 1 Switch on	Switch on the mill.	
Step 2 Preparation	Check if the milling cups and steel balls are clean, if not, clean with compressed air.	
	Put the sample and one metal ball into the milling cup.	
	Put the milling cups in the holder of the ball mill.	

	Tighten the holders with the wheel and close the hood.	
Step 3 Milling	Set the frequency: 20 Hz is a good starting point. Set the time: Usually 2-3 minutes are sufficient. Press the start button. Either wait until the countdown has expired or press the stop button to end the milling manually.	   
	Open the hood, remove the milling cups and take out the ground samples. Clean the milling cups with compressed air.	

3.4.4. Ball mill with adapter for microcentrifuge tubes

Step	Procedure	
Step 1 Switch on	Switch on the mill.	
Step 2 Preparation	Open the lid of the microcentrifuge tube adapter.	
	Put the sample and two metal balls inside the microcentrifuge tube. Note: The samples must be completely dry.	
	Put the tubes into the adapter.	

	Close the adapter so that the bumps are exactly opposite to each other.	
	Put the adapters in the holder of the ball mill and tighten the holders with the wheel.	
Step 3: Set parameters of the mill	Set the frequency. 20 Hz is a good starting point. Set the time. Usually 2-3 minutes are sufficient.	
Step 4: Milling	Press the start button. Either wait until the countdown has expired or press the stop button to end the milling manually.	 
	Remove the adapters from the holder and take out the microcentrifuge tubes with the milled samples.	

4. SAMPLING PLANT MATERIAL THROUGH PUNCHING

4.1. SCOPE AND FIELD OF APPLICATION

The plant sampling method described in this chapter can be used if processes are studied at several growth stages in the same plants, or if isotopic signatures of different plant segments are compared. Typical examples are water stress studies or homogeneity testing of isotope labelling methods.

4.2. BACKGROUND INFORMATION AND CONSIDERATIONS

Because only small amounts of plant material are punched out, this method is only marginally destructive and allows the same plant to be used during the whole experimental process. But sampling only very small amounts implies the risk of not meeting the requirements of a representative sample. Therefore, the variability of isotope ratios within the plant must be assessed in a pre-test by comparing the isotopic ratio of different positions within the plant. The variability can be reduced, if comparable positions at similar growing stages are sampled, e.g. flag leaves during the grain filling stage or last fully developed leaf.

Punched samples can be dried directly in the tin capsules with known tare weight or they can be dried in e.g. multiple well plates and weighted into tin capsules before measurement. The amount of material required for IRMS analysis depends on the dry weight and the C and N content of the material. If the moisture/dry matter ratio of the plant is roughly known, the required number and diameter of punched slices can be determined to meet the amount of fresh material according to the IRMS requirements. The required fresh weight can be calculated using the following equation:

$$FW = \frac{Desired\ C\ amount\ for\ IRMS * 100 * 100}{\%C * (100 - \%\ leaf\ moisture)} \quad (3.1)$$

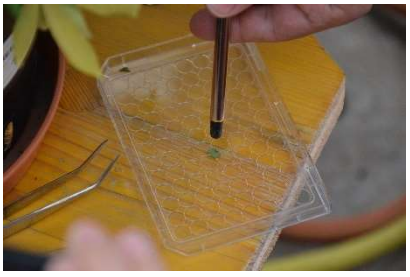
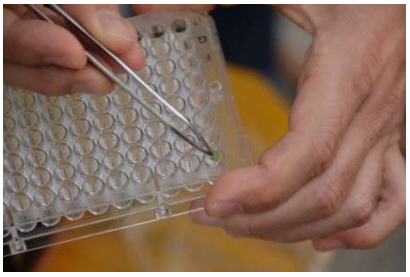
where FW is the fresh weight of the sample (mg), desired C amount for IRMS (mg) depends on the IRMS settings, %C is the percentage carbon in the dry plant material (%) and % leaf moisture is the moisture content of the sample as percentage of the total fresh weight.

Care must be taken if the thickness of the leaves is not homogeneous (for example in sampling various positions in a grass leaf): the same number of punches will not yield the same amount of material for analysis.

4.3. EQUIPMENT

- Punching tool: cork borer or office paper puncher
- Multiple well plate
- Micro balance (readability 0.01 or 0.001mg, depending on target sample weight)
- Oven
- Tin capsules
- Tweezers
- Alcohol to clean tools

4.4. PROCEDURE

Step	Procedure	
Step 1 Punching	Punch a small hole with a cork borer. Alternatively, if a cork borer is not available, a paper hole punch can also be used.	 
	If a cork borer is used: push a rod with a diameter smaller than the borer through and collect the sampled plant material.	
Step 2 Cleaning	Rinse cork borer and rod with ethanol.	
	Transfer the plant material to a clean multiple well plate. Write down the position of the sample. Alternatively, punched samples can be put directly into tin capsules with known tare weight (see 3.2.).	

Step 3 Drying	Dry the samples at 65 ºC and weigh the desired amount into the tin capsule. See equation (3.1)	
Step 4 Preparing for IRMS analysis	Close tin capsule as described in "Weighing of solid samples". Carry out IRMS analyses."	See 4.5.1. Weighing of solid samples, page 18.

5. SAMPLE WEIGHING FOR ISOTOPE RATIO MASS SPECTROMETRY

5.1. SCOPE AND FIELD OF APPLICATION

This SOP covers the weighting and encapsulation of solid soil and plant samples as well as liquid standards and samples into tin capsules. This procedure is essential prior to IRMS analysis. The proposed procedure is relevant to any model of micro balances of any provider.

5.2. BACKGROUND INFORMATION AND CONSIDERATIONS

Plant and soil samples must be milled to a fine powder (less than 0.85mm) prior to weighing. Small samples such as tiny insects and punched leaves can be weighed into tin capsules as a whole.

For target sample weights larger than 1 mg, a micro balance with 0.01 mg readability is sufficient. In case less than 1 mg per sample is weighed, a micro balance with 0.001 mg readability should be used. The correct levelling of the micro balance must be checked every day. Modern micro balances perform calibration automatically during the day, but this does not replace external calibration of the balance. External calibration of micro balances on a regular basis is mandatory for good Quality Management.

Labelling of samples with barcodes further improves Quality Management in the laboratory. A balance and a barcode reader can be connected to a computer, sample barcodes are scanned during the weighing process and sample weights are automatically recorded to an excel file. A system like that speeds up daily routine processes and helps avoiding human errors such as mixing up of samples or recording wrong weights by mistake. An example is shown in: “4.5.3. Optional recording of sample weights using an Excel macro”.

Special attention is needed when working with ^{15}N enriched samples and standards. Best practice is to treat, store and weigh natural abundance and ^{15}N enriched samples separately. In case this is not achievable due to limitations of space and instruments, follow these steps:

- Never treat or weigh natural abundance and enriched samples at the same time.
- Put tin capsules with weighed ^{15}N and natural abundance samples in separate sample trays.
- Clean the micro balance thoroughly with alcohol and all tools with compressed air and alcohol.
- Avoid any extensive dust formation during manipulation and weighing of ^{15}N enriched samples.

5.3. REAGENTS

- Chromosorb P or W (for weighing of liquid standards and samples)

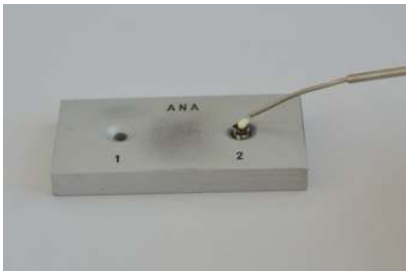
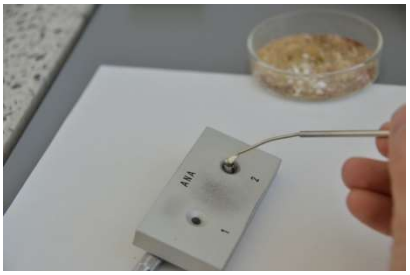
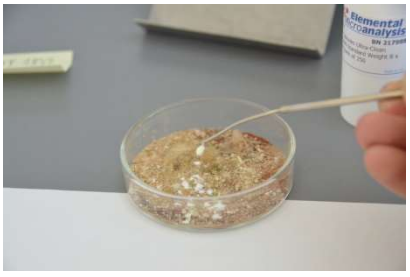
5.4. EQUIPMENT

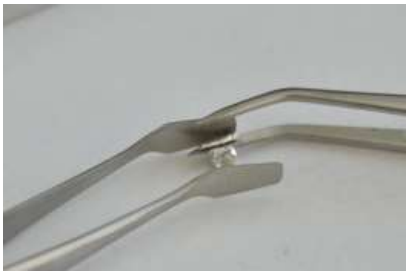
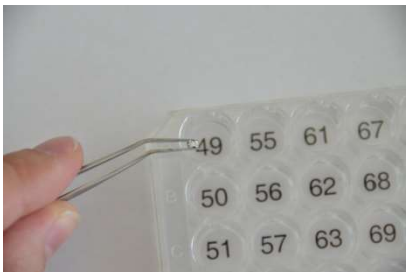
- Micro balance (readability 0.01 or 0.001 mg, depending on target sample weight)
- Tin capsules (ultra clean)
- Tweezers
- Spoon spatula
- Crimping device for capsules
- Crimper plate
- Micro-plate rack with lid

5.5. PROCEDURE

5.5.1. Weighing of solid samples

Step	Procedure	
Step 1 Weighing	Using tweezers, put a tin capsule on the balance (do not touch the tin capsule with your fingers at any stage).	
	Close the door of the balance. Set the balance to zero.	
	Take the tin capsule out with tweezers.	

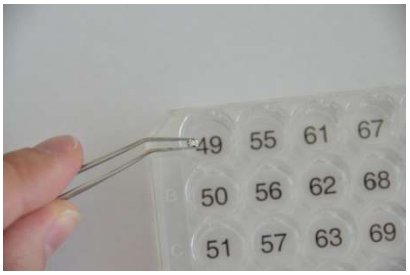
	Place the tin capsule in a crimper plate.	
	Carefully put the sample with the spoon spatula into the tin capsule. Do not contaminate the capsule outside.	
	Put the capsule with the sample on the balance, close the door. Record date, sample name, sample weight and position in the micro-plate rack.	
	In case there is too much sample in the capsule, hold the crimper plate in a slanted position to remove the excess sample material from the tin capsule.	
	Place the excess sample material in a waste container. Do not put the sample back into the original container to avoid contamination.	

Step 2 Crimping	Close the tin capsule with the crimping device.	
	Seal the tin capsule using tweezers. Fold the end of the capsule inwards.	
	Fold the end of the capsule once more.	
	Form a small cube or ball using tweezers. Do not puncture the tin capsule with the tweezers!	
	Place the sample in a labelled micro-plate rack according to the recorded position and close the lid. The sample is now ready for stable isotope analysis.	

Step 3 Cleaning	After finishing the working steps clean the tools with compressed air.	
	Leave the working space clean and orderly.	

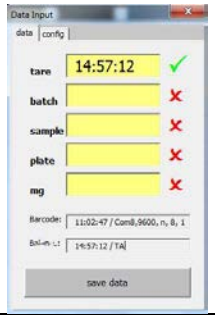
5.5.2. Encapsulation of liquid samples

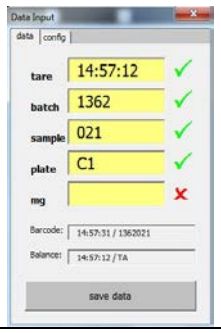
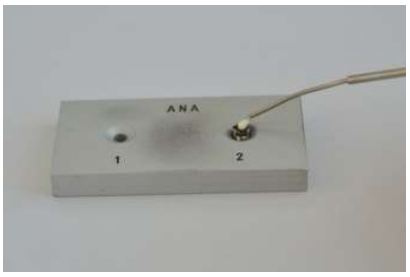
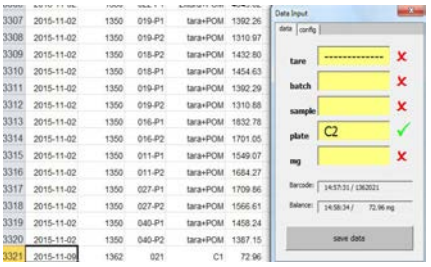
Step	Procedure	
Step 1 Pipetting	Place a tin capsule in the crimper plate. Add approximately 3 to 4mg of chromosorb P or chromosorb W to absorb the liquid sample (a heaped spatula is sufficient).	
	Pipette the sample (maximum 10µl) onto the chromosorb. All liquid needs to be absorbed. Record date, sample name, sample volume and position in the micro-plate rack.	

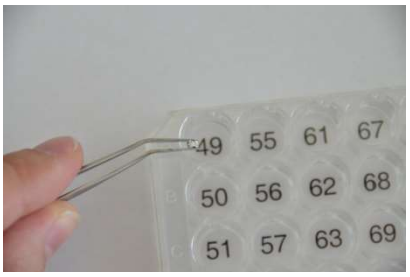
Step 2 Crimping	Close the tin capsule with the crimping device.	
	Seal the tin capsule using tweezers. Fold the end of the capsule inwards. Fold the end of the capsule once more. Form a small cube or ball using tweezers. Do not puncture the tin capsule with the tweezers!	  
	Place the sample in a labelled micro-plate rack according to the recorded position and close the lid. The sample is now ready for IRMS analysis.	

5.5.3. Optional: recording of sample weights using an Excel macro

Step	Procedure	
Step 1 Weighing	Using tweezers, put a tin capsule on the balance (do not touch the tin capsule with your fingers at any stage).	

	Close the door of the balance. Set the balance to zero.	
	Tare weight is automatically transferred to the macro in the Excel sheet.	
	Take the tin capsule out with tweezers.	
	Place the tin capsule in a crimper plate.	
	Scan the sample barcode with the IR-scanner.	

	The scanned data is automatically transferred to the macro in the Excel sheet.	
	Type in the plate number.	
	Carefully put the sample into the tin capsule, using a spoon spatula. Do not contaminate the capsule outside.	
	Put the capsule with the sample on the balance, close the door and press the button “print” on the balance.	
	The values are transferred to the Excel sheet. The macro is ready for weighing of the next sample. The position on the micro-plate rack is automatically counted up to the next position.	

Step 2 Crimping	Close the tin capsule with the crimping device.	
	Seal the tin capsule using tweezers. Fold the end of the capsule inwards. Fold the end of the capsule once more. Form a small cube or ball using tweezers. Do not puncture the tin capsule with the tweezers!	  
	Place the sample in a labelled micro-plate rack according to the recorded position and close the lid. The sample is now ready for IRMS analysis.	
Step 3 Cleaning	After finishing the working steps clean the tools with compressed air.	

6. CARBONATE REMOVAL FROM SOIL FOR DETERMINATION OF ORGANIC CARBON BY ISOTOPE RATIO MASS SPECTROMETRY

6.1. SCOPE AND FIELD OF APPLICATION

This SOP covers the removal of carbonates from soil for determination of organic carbon and its $\delta^{13}C$ signature by IRMS analysis.

6.2. BACKGROUND INFORMATION AND CONSIDERATIONS

Removal of carbonates from calcareous soils is mandatory for analysis of soil organic carbon (SOC) quantity and its $\delta^{13}C$ signature by IRMS. The $\delta^{13}C$ signatures of carbonates are typically in the range of -10‰ to +2‰, while SOC $\delta^{13}C$ signatures vary from about -27‰ to -12‰. As $\delta^{13}C$ signatures of carbonates and SOC differ, even small amounts of remaining carbonates cause considerable errors in the $\delta^{13}C$ signatures of SOC.

Carbonate removal by direct acidification and washing with water leads to losses of acid- and water-soluble fractions of organic carbon and should only be used when larger amounts of soil are required for the analysis or extremely persistent carbonates are present, which cannot be completely removed by acid fumigation.

Acid fumigation is the method of choice for removal of carbonates from soil for IRMS analysis. This method is less time consuming than direct acidification and there is no loss of acid- and water-soluble organic carbon, because these fractions remain inside the silver capsule and are not washed out. The maximum sample weight of dry soil, using 6x4 mm silver capsules is limited to 15mg. Higher sample weights are problematic, because the acid may not reach the soil at the bottom of the silver capsule. However, this 15mg limit is only relevant for soils with extremely low SOC content. For most soils, less than 15mg will suffice for the analysis.

Acidification leads to losses of N and shifts in isotopic signatures of $\delta^{15}N$, therefore N and $\delta^{15}N$ signatures of soils have to be analyzed in two separate aliquots Untreated soil samples have to be analyzed first, to determine the amount of N, $\delta^{15}N$, the amount of total C (carbonates plus SOC) and the corresponding $\delta^{13}C$ (mixed signatures of carbonates plus SOC). Based on the amount of N, the target sample weight for acid fumigation can be estimated by assuming a typical soil C/N ratio of about 10:1 to 15:1. The analysis of the acid fumigated samples will give the amount of SOC (without carbonates) and SOC $\delta^{13}C$ (without carbonates).

6.3. REAGENTS

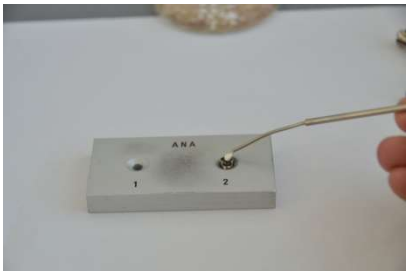
- Distilled water
- Hydrochloric acid, concentrated
- Hydrochloric acid 10% v/v

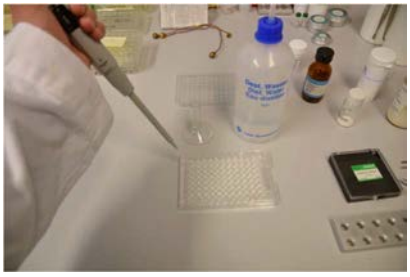
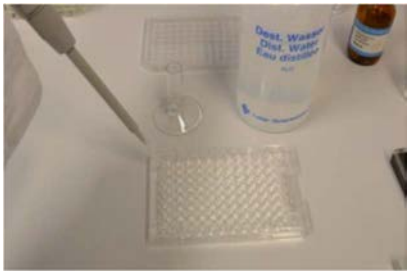
6.4. EQUIPMENT

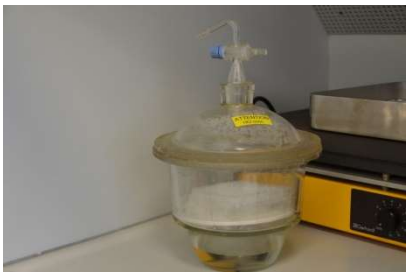
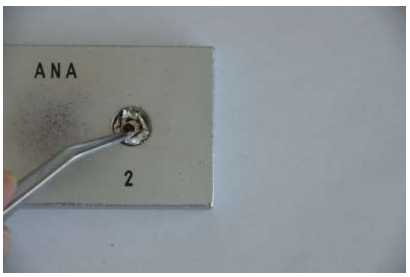
- Micro balance (readability 0.01mg)
- Tin capsules (8x5 mm)
- Silver capsules (6x4 mm)
- Tweezers
- Spoon spatula
- Crimping device for capsules
- Crimper plate
- Micro-well plate, round bottom with lid
- Desiccator
- Test tubes
- Glass fibre filters
- Funnel

6.5. PROCEDURE

6.5.1. Acid fumigation of soils to remove carbonates

Step 1 Weighing	Using tweezers, put a silver capsule on the balance (do not touch the silver capsule with your fingers at any stage).	
	Close the door of the balance, set the balance to zero and take the silver capsule out with tweezers. Place the silver capsule in a crimper plate.	   
	Carefully put the sample with the spoon spatula into the silver capsule. Do not exceed the maximum target sample weight of 15mg soil.	

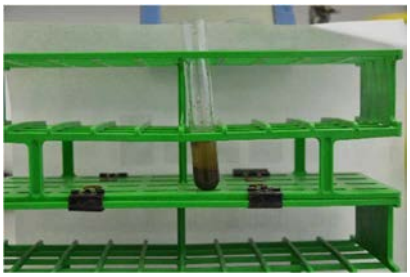
	Put the silver capsule with the sample on the balance, close the door. Record date, sample name, sample weight and position in the micro-well plate.	
	Important: Do not close the silver capsule! Place the open silver capsule in a labelled micro-well plate.	
Step 2 Acid fumigation	Pipette 30µl distilled water onto the sample.	
	Fill the bottom of an empty desiccator with concentrated hydrochloric acid. The hydrochloric acid level shall be below the screen plate. Place the labelled micro-well plate into the desiccator with concentrated hydrochloric acid for at least 6 hours. Caution: follow safety guidelines, label desiccator accordingly and work in a fume hood.	
	Remove the micro-well plate from the desiccator and pipette another 30µl distilled water on the sample. Caution: hydrochloric acid has condensed on the micro-well plate, so wear gloves when you handle the plate!	

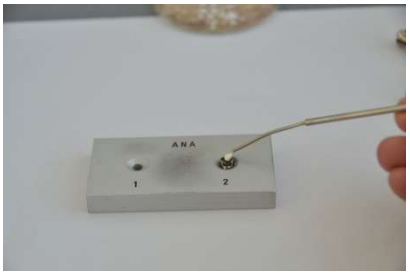
	Place the labelled micro-well plate back into the desiccator with concentrated hydrochloric acid for another 6 hours.	
	Place the labelled micro-well plate inside a drying oven at 60°C for at least 6 hours.	
	Put one tin capsule in a crimper plate (do not touch the tin capsule with your fingers).	
	Put the silver capsule containing the dried fumigated sample inside the tin capsule (do not touch the capsules with your fingers).	
	Close the tin capsule with the crimping device and seal the tin capsule using tweezers. Fold the end of the capsule inwards. Fold the end of the capsule once more. Form a small cube or ball using tweezers. Do not puncture the tin capsule with the tweezers!	   

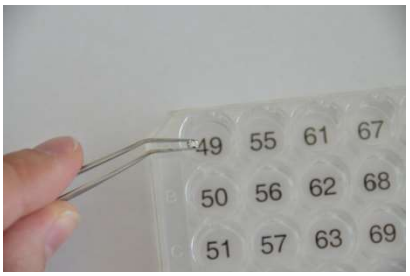
	Place the sample in a labelled micro-well plate according to the recorded position and close the lid. The sample is now ready for IRMS analysis.	
Step 3 Cleaning	After finishing the working steps, flush the used tools with compressed air.	
	Leave the working space clean and orderly.	

6.5.2. Direct acidification of soils to remove carbonates

Step 1 Acidification	Put about 1 g dry soil sample, ground into a fine powder, into a test tube.	
	Pour about 2 ml hydrochloric acid (10%) into the test tube. Wait until the fizzling stops, then add further 1 ml aliquots of hydrochloric acid (10%) until addition of more acid does not produce any fizzing.	

	Leave the sample with the hydrochloric acid (10%) in the test tube for at least 12 hours to “digest”.	
Step 2 Filtering	Pour the mixture onto a glass fibre filter.	
	Pour about 4ml of distilled water to the residues in the test tube.	
	Pour the residues onto the filter cake. Repeat this step, until all the residues from the test tube are transferred onto the filter.	
Step 3 Drying	Dry the filter containing the residue at 40°C for at least 12 hours.	

Step 4 Weighing	Put a tin capsule on the balance (do not touch the tin capsule with your fingers).	
	Close the door of the balance, set the balance to zero and take the tin capsule out with tweezers. Place the tin capsule in a crimper plate.	   
	Carefully put the sample with the spoon spatula into the tin capsule. Do not contaminate the capsule outside.	
	Put the capsule with the sample on the balance, close the door. Record date, sample name, sample weight and position in the micro-plate rack.	
Step 5 Crimping	Close the tin capsule with the crimping device and seal the tin capsule using tweezers. Fold the end of the capsule inwards. Fold the end of the capsule once more. Form a small cube or ball using tweezers. Do not puncture the tin capsule with the tweezers!	   

	Place the sample in a labelled micro-plate rack according to the recorded position and close the lid. The sample is now ready for stable isotope analysis.	

BIBLIOGRAPHY

BRODIE, C.R., LENG, M.J., CASFORD, J.S.L., KENDRICK, C.P., LLOYD, J.M., YONGQIANG, Z. and BIRD, M.I., Evidence for bias in C and N concentrations and $\delta^{13}C$ composition of terrestrial and aquatic organic materials due to pre-analysis acid preparation methods, Chem. Geol. **282** (2011) 67–83.

FERNANDES, M., and KRULL, E., How does acid treatment to remove carbonates affect the isotopic and elemental composition of soils and sediments? Environ. Chem. **5** (2008) 33–39.

HARRIS, D., HORWÁTH, W.R., and VanKESSEL, C., Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis, Soil Sci. Soc. Am. J. **65** (2001) 1853.

HEDGES, J., and STERN, J., Carbon and nitrogen determinations of carbonate-containing solids, Limnol. Oceanogr. **29** (1984) 657–663.

KENNEDY, P., KENNEDY, H., and PAPADIMITRIOU, S., The effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment, Rapid Commun. Mass Spectrom. **19** (2005) 1063–1068.

KOMADA, T., ANDERSON, M.R., and DORFMEIER, C.L., Carbonate removal from coastal sediments for the determination of organic carbon and its isotopic signatures, $d^{13}C$ and $\Delta^{14}C$: comparison of fumigation and direct acidification by hydrochloric acid. Limnol. Oceanogr. Methods **6** (2008) 254–262.

CONTRIBUTORS TO DRAFTING AND REVIEW

Gruber, R.	International Atomic Energy Agency
Heiling, M.	International Atomic Energy Agency
Resch, C.	International Atomic Energy Agency
Slaets, J.	International Atomic Energy Agency

IAEA
International Atomic Energy Agency

No. 25

ORDERING LOCALLY

In the following countries, IAEA priced publications may be purchased from the sources listed below or from major local booksellers.

Orders for unpriced publications should be made directly to the IAEA. The contact details are given at the end of this list.

CANADA

Renouf Publishing Co. Ltd

22-1010 Polytek Street, Ottawa, ON K1J 9J1, CANADA
Telephone: +1 613 745 2665 • Fax: +1 643 745 7660
Email: order@renoufbooks.com • Web site: www.renoufbooks.com

Bernan / Rowman & Littlefield

15200 NBN Way, Blue Ridge Summit, PA 17214, USA
Tel: +1 800 462 6420 • Fax: +1 800 338 4550
Email: orders@rowman.com Web site: www.rowman.com/bernan

CZECH REPUBLIC

Suweco CZ, s.r.o.

Sestupná 153/11, 162 00 Prague 6, CZECH REPUBLIC
Telephone: +420 242 459 205 • Fax: +420 284 821 646
Email: nakup@suweco.cz • Web site: www.suweco.cz

FRANCE

Form-Edit

5 rue Janssen, PO Box 25, 75921 Paris CEDEX, FRANCE
Telephone: +33 1 42 01 49 49 • Fax: +33 1 42 01 90 90
Email: formedit@formedit.fr • Web site: www.form-edit.com

GERMANY

Goethe Buchhandlung Teubig GmbH

Schweitzer Fachinformationen
Willstätterstrasse 15, 40549 Düsseldorf, GERMANY
Telephone: +49 (0) 211 49 874 015 • Fax: +49 (0) 211 49 874 28
Email: kundenbetreuung.goethe@schweitzer-online.de • Web site: www.goethebuch.de

INDIA

Allied Publishers

1st Floor, Dubash House, 15, J.N. Heredi Marg, Ballard Estate, Mumbai 400001, INDIA
Telephone: +91 22 4212 6930/31/69 • Fax: +91 22 2261 7928
Email: alliedpl@vsnl.com • Web site: www.alliedpublishers.com

Bookwell

3/79 Nirankari, Delhi 110009, INDIA
Telephone: +91 11 2760 1283/4536
Email: bkwell@nde.vsnl.net.in • Web site: www.bookwellindia.com

ITALY

Libreria Scientifica "AEIOU"

Via Vincenzo Maria Coronelli 6, 20146 Milan, ITALY
Telephone: +39 02 48 95 45 52 • Fax: +39 02 48 95 45 48
Email: info@libreriaaeiou.eu • Web site: www.libreriaaeiou.eu

JAPAN

Maruzen-Yushodo Co., Ltd

10-10 Yotsuyasakamachi, Shinjuku-ku, Tokyo 160-0002, JAPAN
Telephone: +81 3 4335 9312 • Fax: +81 3 4335 9364
Email: bookimport@maruzen.co.jp • Web site: www.maruzen.co.jp

RUSSIAN FEDERATION

Scientific and Engineering Centre for Nuclear and Radiation Safety

107140, Moscow, Malaya Krasnoselskaya st. 2/8, bld. 5, RUSSIAN FEDERATION
Telephone: +7 499 264 00 03 • Fax: +7 499 264 28 59
Email: secnrs@secnrs.ru • Web site: www.secnrs.ru

UNITED STATES OF AMERICA

Bernan / Rowman & Littlefield

15200 NBN Way, Blue Ridge Summit, PA 17214, USA
Tel: +1 800 462 6420 • Fax: +1 800 338 4550
Email: orders@rowman.com • Web site: www.rowman.com/bernan

Renouf Publishing Co. Ltd

812 Proctor Avenue, Ogdensburg, NY 13669-2205, USA
Telephone: +1 888 551 7470 • Fax: +1 888 551 7471
Email: orders@renoufbooks.com • Web site: www.renoufbooks.com

Orders for both priced and unpriced publications may be addressed directly to:

Marketing and Sales Unit
International Atomic Energy Agency
Vienna International Centre, PO Box 100, 1400 Vienna, Austria
Telephone: +43 1 2600 22529 or 22530 • Fax: +43 1 2600 29302 or +43 1 26007 22529
Email: sales.publications@iaea.org • Web site: www.iaea.org/books

International Atomic Energy Agency
Vienna
ISBN 978–92–0–102019–2
ISSN 1011–4289