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A procedure of determining carbon-13 composition in soil organic carbon on an Isotope Ratio Mass-Spectrometer

Nguyen Thi Hong Thinh[1], Vu Hoai[1], Ha Lan Anh[1], Trinh Van Giap[1], Nguyen Van Vuong[2]

[1] Isotope Hydrology Laboratory, Institute for Nuclear Science and Technology
179 Hoang Quoc Viet str., Cau Giay dist., Hanoi, Vietnam
[2] Hanoi University of Natural Science, Vietnam National University, 254 Nguyen Trai, Hanoi
Email: nhthinh2001@yahoo.com

Abstract: In this study, a procedure of determining the ^{13}C isotope composition ($[^{13}C]/[^{12}C]$) in soil organic carbon (SOC) using an isotope ratio mass spectrometer (IRMS) was developed. The procedure would be a useful approach in the studies on carbon sequestration that is of great concern among environmentalists worldwide nowadays. The procedure includes: drying, crushing, sifting and removing carbonate in soil samples before the analysis on the mass spectrometer. Results showed that the developed procedure gained a good repeatability of 0.21‰. The accuracy of the procedure was checked by analyzing a surrogate soil sample, a mixture of soil with known $\delta^{13}C_{SOC}$ and IAEA-CH-3 cellulose standard.

Keywords: *Soil organic carbon, $^{13}C/^{12}C$ isotope ratio, isotope ratio mass spectrometer, EA- IRMS.*

I. INTRODUCTION

In soil science, soil organic carbon (SOC) plays a very important role in creation of soil structure, soil chemical and physical characteristics and soil fertility, etc. Stable isotope ratio of $[^{13}C]/[^{12}C]$ in the SOC as it was expressed in the delta notation ($\delta^{13}C_{SOC}$) - a natural tracer, is interested in many areas of research on environmental processes such as carbon sinks and photosynthetic mechanisms of plants [1], assessing the carbon reservoir turnover times and soil carbon dynamic in agroforestry ecosystems, methods of fixation and storage of carbon dioxide in soils [2, 3, 4, 5, 6] or exploring soil mineralization processes [7]. For getting accurate and reliable $\delta^{13}C_{SOC}$ analysis results, laboratories will need to convey and apply suitable methods of treatment and analysis for soil samples. Carbon in the soil exists in two main forms: inorganic carbonate (IC) and organic carbon (OC), and they have different $\delta^{13}C$ values. When analyzing the C-13 isotope composition of the SOC, it is necessary to eliminate the IC component completely. Inorganic acids are used to remove carbonate in the soil. There are three most comment of acid treatment ways to remove the IC for $\delta^{13}C_{SOC}$ analysis: simple acidification, capsule and fumigation method [8, 9, 10, 11, 12]. Each method has its own advantages and disadvantage for soil samples, but the fumigation method has more advantages for agricultural soil samples treatment [10].

The objective of this study was to develop a procedure for accurately determining $\delta^{13}C_{SOC}$ in soil on an Isotope Ratio Mass-Spectrometer equipped with an Elemental Analyzer (EA-IRMS) at the Isotope Hydrology Lab – INST. The procedure developed will be assessed with its repeatability as well as its accuracy.

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II. MATERIALS AND METHODS

A. Material

Soil samples were collected at a cultivated land in Dan Phuong (21°06'21.0" N, 105°39'45.0" E) and Dong Anh (21°10'19.0" N, 105°47'26.2"E) districts – a suburban area of Hanoi city. The soils are alluvial on which dominant crops such as rice, maize are cultivated. The soil samples were taken using a core sampler (6 cm i.d.) to a depth of 30 cm and then it was divided into two layers: 0-15 cm and 15 - 30 cm depth. The samples were spread on stainless steel trays using a stainless-steel spatula to dry at room temperature or at 40°C - 50°C in a ventilated oven for two days. The dried soils were homogenized using ceramic mortar and then sieved through 1 mm mesh sieve to remove bricks, stones, gravel and roots. The samples were then ground and sieved through 100 μm mesh sieve, the dried at 50°C for 24 hours. Finally, the samples were subdivided into subsamples with 30 – 40 mg each prior removing the IC and analysis for the $\delta^{13}C_{SOC}$.

B. Removing carbonates in soils

Before the IC removing, the concentration of total soil carbon and soil organic carbon were determined by the TCVN 6642: 2000 method to estimate an appropriate quantity of soil sample needed for the next carbonate treatment step. The fumigation method was used in this study to remove the IC in the soil samples. The method employs *in-situ* acidification that could avoid preferential loss of soluble organic material during the treatment which would be happened in the rinse method [13, 14].

Soil subsamples of (30-40) mg from the 0-15 cm and 15-30 cm depths were weighted into 2ml glass vials, placed in a multi wells plastic tray and moistened with 50 μl of de-ionized water. The tray was then placed into a vacuum desiccator of 5 L capacity together with a beaker containing 100 mL of 12M HCl. The desiccator was air evacuated for 5 minutes, and then locked by the suction valve. The soil samples were exposed to HCl vapor for 3h, 6h, 12h and 24h to investigate the optimum fumigation time.

After each fixed time of fumigation, the HCl beaker was taken out and the desiccator was air-evacuated again for 1-1.5h to remove all acid vapors. The samples were dried at 60°C for 12 hours, cooled in a desiccator, grounded by glass rod and then tightly caped. The treated soils were weighed with an amount that would contain (60-80) μg (±2) μg of the OC then wrapped into tin capsules. The capsules were loaded into an auto-sampler of the analytical equipment.

C. Determination of $\delta^{13}C_{SOC}$ by EA-IRMS

The ^{13}C isotope composition in soil samples were analyzed using an Isotopes Ratio Mass Spectrometer (IR MS, Micromass GV Instrument, UK) equipped with an Elemental Analyzer (EuroVector, Italy) at the Isotopes Hydrology Laboratory, Institute for Nuclear Sciences and Technology, INST (VINATOM) as shown in Figure 1.

Fig.1. The EA-IRMS system at the Isotopes Hydrology Laboratory, INST (VINATOM)

The organic carbon in the soil samples was oxidized at 1030°C to produce CO_2, NO_x gases and H_2O in the combustion reactor of the EA in which the chromium oxide catalyst and cobaltous silver oxide was packed. Continuous flow of helium will carry these gases through a reduction reactor containing high purity copper wires to reduce NO_x into N_2 gas and remove excess oxygen at 650°C. The water was entraped in a “water trap” containing magnesium perchlorate. Finally, CO_2 and N_2 gases were separated from each other *via* a packed chromatographic column and then entered the ionization chamber of the IRMS. In the ionization chamber, CO_2 will be ionized to form CO_2^+ ions following the separation by its mass numbers 44 and 45 corresponding to $^{12}CO_2$ and $^{13}CO_2$. The intensity of the mass peaks was recorded by the Faraday cups installed next to the magnetic mass separator. The information generated by mass peaks will be analyzed by the software supplied by the GV supplier.

The $^{13}C/^{12}C$ isotope ratio in the OC is expressed in the delta notation ($\delta^{13}C$) as follows:

$$\delta^{13}C\ (‰) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right)*1000$$

Where:

R_{sample} is the mole ratio of the $[^{13}C]/[^{12}C]$ in the sample;

R_{standard} is the mole ratio of the $[^{13}C]/[^{12}C]$ in the standard.

The standard used for this analysis is Vienna Pee Dee Belemnite (VPDB) supplied by the International Atomic Energy Agency (IAEA) in Vienna, Austria.

D. The repeatability and accuracy of the method

Before running the samples on the mass spectrometer, the IR MS was checked for its stability and linearity using CO_2 ultrapure gas (99,999%) supplied by the Viet-Nhat gas company. According to the guide of the IR MS supplier, the equipment could be considered to work stable if the standard deviation from ten 45/44 mass ratios of the 10 consecutive analyses for the same gas sample were less than 0.5‰. The IR MS system could be considered to have a good linearity if a graph of 45/44 mass ratio obtained from 10 current intensities in the range from 2 to 12 nA showed a correlation coefficient (R^2) better than 0.99.

The accuracy of the measurement was controlled by using of three reference standards CO-9 ($\delta^{13}C_{VPDB}$: -47.1‰); IAEA CO-8 ($\delta^{13}C_{VPDB}$: -5.75‰) and IAEA-CH-3 ($\delta^{13}C_{VPDB}$: -22.72 ‰) which were supplied by the IAEA.

The repeatability and accuracy of the developed method was tested 10 times with a random soil sample. The procedure was as follows:

A soil sample was fumigated and measured for its $\delta^{13}C_{SOC}$ which showed to have 1% SOC and $\delta^{13}C_{SOM}$ of -(21.02 ± 0.21) ‰. Then 3,378 mg of the IAEA-CH-3 cellulose standard having 44, 41% C and $\delta^{13}C$ of -(24.72 ± 0.04)‰ was added to 150 mg of this soil sample. The fumigation and analytical procedure for the $\delta^{13}C_{SOC}$ were repeated for the surrogate samples.

III. RESULTS AND DISCUSSION

A. The repeatibility and linearity of the EA-IR MS

Results of the analysis for the $\delta^{13}C$ in the Viet-Nhat ultrapure CO_2 gas showed a repeatibility of better than 0.3 ‰. The signal of the 45 to 44 mass ratios in different amounts of the IAEA-CH-3 ($\delta^{13}C_{VPDB}$: -22.72‰) that generated currents in a range of 2 to 12 nA showed a good linearity with a R^2 = 0.999.

B. The optimum fumigation time

Two soil samples at 2 depths (0-15) cm

and (15-30) cm containing the highest inorganic carbon content, up to 0.4% were chosen to monitor the change in $\delta^{13}C$ value over time of the acid fumigation. The results of this study were shown in Fig. 2 and Fig. 3.

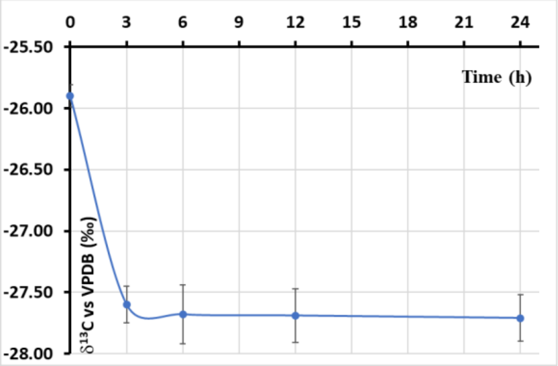

Fig. 2. The variation of $\delta^{13}C$ vs VPDB in soil samples at (0-15) cm layer over time of HCl acid fumigation.

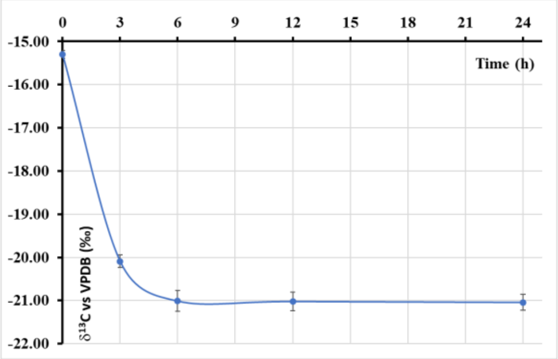

Fig. 3. The variation of $\delta^{13}C$ vs VPDB in soil samples at 15 – 30 cm layer over time of HCl acid fumigation

Results in Fig.2 showed that the average $\delta^{13}C$ in untreated soil sample at (0-15) cm depth was depleted from – (25.9 ± 0.09)‰, (n = 9) and became unchanged at –(27.69±0.22)‰ after a period of 6h to 24h fumigation. The $\delta^{13}C$ in untreated soil sample at the (15-30) cm depth was also depleted from – (15.30 ± 0.12)‰, (n=9) to -(21.02 ± 0.21)‰ after 6h to 24h of acid fumigation (Fig.3). Therefore, 6h was decided to be an optimum time for the acid removal of the IC in the soils at the both depths.

It was reported that the time needed to decompose 2.4% of IC in 30 mg of soil was 6h and the decomposition rate was dependent on the IC content in each sample as well as the amount of diffused soil [13]. In this study, the amount of diffused soil sample also was 30 mg, but the IC content was 0.1% to 0.3%, corresponding to 0.03 mg and 0.09 mg IC in soils at 0-15 cm and 15-30 cm depths, respectively. Apparently, the rate of the carbonate removal in this study was slower than that of the study in the reference [13]. This might be due to the glass vials used in this study as containers for soils in the fumigation process did not facilitated the acid vapor to diffuse in the soil samples. In the Harris study [13] silver capsules containers were used so it could much improve the HCl vapor diffusion. However, the use of glass vials has an advantage than capsules as it could reduce the amount of ash (silver) deposited on the reaction column that avoids the risk of blocking the column during the analysis.

C. The repeatability and accuracy of the procedure

The carbon-13 composition in the SOC ($\delta^{13}C_{SOC}$) of a soil sample at the (15-30) cm depth was determined following the fumigation treatment and EA-IRMS analysis with 10 replicates. The results of the test were presented in Table I.

Table I: Repeatability of the $\delta^{13}C_{SOC}$ in a soil sample at (15-20) cm depth that was derived from the 6h HCl fumigation and EA-IRMS analysis

Test No.	$\delta^{13}C_{SOC}$ vs. VPDB, ‰
Test soil 1	-20.80
Test soil 2	-21.03
Test soil 3	-20.75
Test soil 4	-21.32

Test soil 5	-21.04
Test soil 6	-21.24
Test soil 7	-20.81
Test soil 8	-21.27
Test soil 9	-21.12
Test soil 10	-20.85
Average	-21.02
Stdev (S_R)	0.21

The results presented in Table I show the repeatability (S_R) of the procedure to be better than 0.3‰.

Table II shows the results of the $\delta^{13}C_{SOC}$ in the surrogate soil sample that has the carbon-13 composition of -22.87‰ *vs*. VPDB.

Table II. The accuracy of the $\delta^{13}C_{SOC}$ determination for a surrogate sample (soil + IAEA CH-3 cellulose standard)

Test No.	**δ^{13}C vs. VPDB, ‰**
Surrogate soil 1	-22.52
Surrogate soil 2	-22.64
Surrogate soil 3	-22.58
Surrogate soil 4	-22.80
Surrogate soil 5	-22.85
Surrogate soil 6	-22.75
Surrogate soil 7	-22.74
Surrogate soil 8	-22.87
Surrogate soil 9	-23.10
Surrogate soil 10	-23.15
δ^{13}C mean	-22.80
Stdev (S_R)	0.21
δ^{13}C assigned value	-22.87
Bias (Δ)	0.07

The data in Table II showed that the average δ^{13}C in the surrogate soil has a good accuracy with a bias of 0.074‰ or 0.4% deviation compared to the assigned value of -22.87‰.

IV. CONCLUSIONS

The conditions for the acid fumigation of soils samples were developed to determine the $\delta^{13}C_{SOC}$ on an isotope ratio mass spectrometer (EA-IRMS). Fumigation by 12M HCl in 6 hours can completely decompose the IC with a low content (<1%) presented in soil samples at depth up to 30 cm from the surface. The developed procedure has a good repeatability of better than 0.3‰ and a bias (accuracy) of (0.4-0.5)% from the standard.

This procedure will be applied in the agricultural environment studies in future.

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