Liquid chromatography/mass spectrometry stable isotope analysis of dissolved organic carbon in stream and soil waters
Patrick Albéric

To cite this version:

HAL Id: insu-00619695
https://hal-insu.archives-ouvertes.fr/insu-00619695
Submitted on 23 May 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
LC/MS stable isotope analysis of dissolved organic carbon in stream and soil waters

Patrick Albéric
Université d'Orléans-CNRS/INSU UMR 6113, Institut des Sciences de la Terre d'Orléans (ISTO) 1A rue de la férollerie, 45071 Orléans cedex2, France.
patrick.alberic@univ-orleans.fr

A commercial interface coupling liquid chromatography (LC) to a continuous-flow isotope ratio mass spectrometer (IRMS) was used to analyse δ¹³C of dissolved organic carbon (DOC) in natural waters. Stream and soil waters from a farmland plot in a hedgerow landscape were selected for application. Based on wet chemical oxidation of dissolved organics the LC-IRMS interface allows on-line injection of small volumes of water samples, oxidation reaction to produce CO₂ and gas transfer to the IRMS. In flow injection analysis (FIA) mode, bulk DOC δ¹³C analysis was performed on up to 100 µL aqueous sample in the range of DOC concentration in fresh waters (1-10 mg C.L⁻¹). Mapping DOC δ¹³C spatial distribution at the plot scale was made possible by this fairly quick method (10 min for triplicate) with little sample manipulation. The relative contributions of different plot sectors to the DOC pool in the stream draining the plot were tentatively inferred on the basis of δ¹³C differences between hydrophilic and hydrophobic components.

key words: DOC δ¹³C, soilwater, LC-IRMS

Several methods coupling a dissolved organic carbon (DOC) analyzer to a continuous flow isotope ratio mass spectrometer (IRMS) have been developed.¹,²,³,⁴,⁵,⁶ As for quantitative DOC analysis, two types of methodologies can achieve organic carbon oxidation to CO₂:¹,² (i) High temperature combustion (HTC) systems with cryogenic trapping before transfer to IRMS.⁴,⁵,⁶ Refractory organic carbon may be better oxidized by HTC systems but isotopic values have to be corrected for a significant instrument blank depending on the volume of water injected and time of cryotrapping.⁴,⁵,⁶ (ii) Wet chemical oxidation (WCO) methods apply to larger sample volumes with variable persulphate reagent concentration in a closed reactor.¹,²,³ Blank corrections also need to be accounted for, mostly depending on reagent
Both types of system can use reduction ovens and gas chromatographic separation before transfer to IRMS. The necessary complete purging of dissolved inorganic carbon (DIC) before DOC (more exactly non-purgeable organic carbon NPOC) analysis has to be done off-line by acid introduction in the sample before injection in HTC systems\textsuperscript{[5,6]} but can be conducted on-line before introducing the oxidation reagent in the reactor for WCO systems, allowing in this case both DIC and DOC carbon isotopic analysis.\textsuperscript{[1,2,3]}

In this work a commercial liquid chromatography (LC)-IRMS interface (LC IsoLink, Thermo Scientific, Bremen, Germany) was used to analyse the variation in DOC $\delta^{13}$C in stream and soil waters. The device, which was specially developed for on-line compound specific isotope analysis of water-soluble samples after LC separation\textsuperscript{[7]}, can also be also used without column in bulk mode analysis for simple\textsuperscript{[7]} as well as for macromolecular compounds.\textsuperscript{[8]} In the field of complex raw samples the LC IsoLink interface has been already used for bulk carbon isotope ratio determination in alcoholic beverages.\textsuperscript{[9]} The main advantage of this method is that the water sample is injected into a continuous flow of mobile phase (ultrapure deionised water) and reagents mixed at the entry of a heated capillary reactor connected on-line to an open-split coupling and the IRMS via a liquid/gas (water/He) membrane exchanger and a gas-drying unit (see Krummen et al.\textsuperscript{[7]} for a detailed description).

The field study selected as an example for application was a farmland plot in which several sectors had been previously distinguished for their different characteristics (redox conditions, ionic strength, dissolved metals, water-table depth).\textsuperscript{[10]} Factors controlling DOC in soils are complex and can be influenced by numerous parameters and processes such as substrate quality, decomposer community, adsorption in mineral soil horizons, pH, ionic strength, temperature, soil moisture, water fluxes and redox conditions.\textsuperscript{[11,12]} Some of these factors may also control the variation in the isotope composition of organic carbon in soil profiles. Rather than direct isotope fractionation mechanisms for which evidence is lacking,\textsuperscript{[13,14]} the effects of selective sorption and/or preferential preservation of isotopically and chemically heterogeneous complex soil organic matter have been investigated, mostly through experiment.\textsuperscript{[15,16,17]} Mapping DOC $\delta^{13}$C in soil water at the plot scale can be a complementary approach to decomposition or sorption experiments but will involve managing a large number of water samples. The aim of the present work was first to test the applicability of the LC IsoLink interface to such a soil water field study. As soils account for the principal source of DOC in streams,\textsuperscript{[18]} the isotope composition of several organic fractions extracted from the water of the small creek draining the plot were compared to the soil water DOC obtained from the different sectors in the plot. Descriptions of the site, soil
horizons, and procedures of sampling or isolation of specific organic fractions (with XAD resins) preliminary to δ¹³C analysis will not be detailed in this paper. The objective was to describe the feasibility of analysing a large number of samples for DOC δ¹³C with small sample volumes at fresh water natural concentrations with a commercial LC-IRMS interface.

MATERIALS AND METHODS

Site and soil description and water sampling
The site and field work have been detailed elsewhere. Briefly, the plot selected for the study is located on a hillside used as pasture in a hedgerow landscape. The soils are Planosols developed on altered gneiss and exhibit a stony horizon rich in Fe–Mn oxides overlaying a clayey B horizon supporting a seasonal water table in winter. Soil waters were collected below the water table from 80 cm depth to near the surface, free-flowing in auger-drilled holes. The volumes of water filling the auger holes were comprised between 0.1 and 2 liters.

Landscapes marked by hedgerows (bocage landscape) are typically non-uniform areas in which the 3D distribution of soil horizons and anthropogenic structures (e.g. hedges, ditches or wheel ruts) affects the composition and circulation of water. Based on water solute composition the plot was found to be subdivided into four geochemical sectors (see Fig. 6 at the end of the paper): (i) A sector lined by hedges characterized by a deeper water table, higher dissolved oxygen content and ionic strength; (ii) a sector with higher DOC concentrations (green grass symbol in Fig. 6) spatially correlated with shallow soil water levels enhancing contact between soil solution and humic top soil horizons; (iii) a sector influenced by the upward infiltration of underground waters rich in reduced manganese in locations where the deep clayey B horizon is thinner or missing; and finally (iv) 2 anoxic Fe²⁺ and Mn²⁺ rich spots closed to the bank of the stream. Each sector may contribute differently to the chemical composition of the stream water.

Sample preparation
Soil water subsamples for DOC analysis were filtered (0.45 μm) and acidified to pH 1-2 either with concentrated nitric or phosphoric acid (but not HCl so as to preserve persulphate oxidation efficiency, see below).

Separation of the different DOC fractions was made on larger volumes of water (about 7 L) sampled in the stream draining the plot. Hydrophobic (HPO) and transphilic (TPH) acids were recovered by tandem XAD8/XAD4 adsorption chromatography. HPOacid and
TPH acid fractions were adsorbed at pH 2 and then eluted in a small volume (50 mL) of basic solution (pH 12) respectively from the XAD8 and the XAD4 resins (80 mL resin bed each). TPH acids are more hydrophilic than HPO acids. Other classically defined fractions\textsuperscript{[22]} either remained in solution in the water passing through the tandem columns (i.e. the most hydrophilic compounds HPI) or were retained on the XAD8 resin (i.e. the more hydrophobic neutral compounds HPO neutral). In addition, analytical separation using only a small XAD8 column\textsuperscript{23} yielded 50% of the HPO acid and 30% of the HPI+TPH.

**DOC $\delta^{13}$C LC-IRMS analysis**

A system composed of an LC unit (Surveyor MS-pump with autosampler) coupled to a DeltaV-Advantage IRMS via a LC-IsoLink interface (all supplied by Thermo Scientific, Hanna-Kunath-Straße 11, Bremen, Germany) was used. Bulk analyses of DOC $\delta^{13}$C were run in flow injection analysis (FIA)\textsuperscript{[8]} mode (i.e. without column). Per mil (‰) deviations from the international standard Vienna PeeDee Belemnite (VPDB) were calculated by the software Isodat (Thermo Scientific). $\delta^{13}$C standardization was done using IAEA (International Atomic Energy Agency) and USGS (US-Geological Survey) simple molecules and complex aquatic fulvic acids from IHSS (International Humic Substances Society).

Mobile phase (MilliQ water brought to pH 2 with H$_3$PO$_4$) and reagents (see Fig. 1 caption) were degassed under vacuum in a sonic bath and then permanently purged with He to prevent air regassing.\textsuperscript{[8,9]} The LC flow rate was fixed at 300µL/min considering its influence on the signal intensity and $\delta^{13}$C values.\textsuperscript{8} Flow rates were fixed at 50µL/min for the acid reagent and between 35 and 40 µL/min for the oxidant reagent to maintain the signal for O$_2$ (m/z 32) at about 10 volts which is indicative of a sufficient level of oxidant through the reactor. The reactor temperature was set at 100°C. A sample volume up to 100 µL was routinely used to lower the detection limit to 0.5 mg C L$^{-1}$. Acidified samples were manually purged with He (for DIC-CO$_2$ elimination) in the autosampler 1.8 mL vials (during 3-4 min) prior to being place in the autosampler rack and injection. For organic concentrated fractions (HPO acid, TPH acid), 10 µL of sample diluted ten-fold with water were used. Each run started with 3 gas pulses for $\delta^{13}$C determination and ended with 2 for control; analysis of the sample in triplicate was completed in 10 minutes (Fig. 1).

*Precision and accuracy of the system*

$\delta^{13}$C values were calculated relative to pulses of CO$_2$ analysed at the beginning of each run. Standard deviation (SD, 1σ) for triplicate analysis of standard solutions or water samples was
less than 0.1‰. The δ¹³C value of the CO₂ monitor gas was repeatedly calibrated over a set of reference standard compounds dissolved in water. The set comprising both simple molecules and natural complex macromolecules ranged from -10.45‰ (sucrose IAEA-CH6) to -27.6‰ (Suwannee River fulvic acid IHSS-1S101F). Intermediate δ¹³C values may be found with sucrose IAEA-C6, -10.8‰; oxalic acid IAEA-C8, -18.3‰ and glutamic acid USGS-40, -26.39‰. The stability over time of the system was controlled by plotting the reference standard δ¹³C values versus measured values. In the example shown in Fig. 2, the maximum difference between targeted and measured values did not exceed 0.5‰. Least-squares linear regression of the data can be used to correct measured values within a 0.3‰ difference to standard values.

Influence of acidification and purging - Reproducibility
Purging DIC before analysis is an obligatory step when measuring measure DOC δ¹³C in natural waters.⁷ In view of the principle of in-flow injection of the water sample in the mobile phase on-line the reactor and the gas exchanger, a simple method consisted in acidifying and purging samples before injection (NPOC treatment). Depending on the alkalinity of the samples, 1 or 2 drops of concentrated phosphoric acid or nitric acid (Merck Suprapur®) were used per 5 mL of water sample. The influence of purging time was tested on MilliQ solutions of Suwannee River fulvic acid and K-biphtalate (Merck Standard compound for elementary analysis used for DOC quantification) and a natural soil water sample, all acidified with phosphoric acid (Fig. 3). Purging was done manually in the 1.8 mL autosampler vials with a flux of He (derived from the reagents and mobile phase purging lines) introduced with a stainless steel syringe needle through the vial septum. As shown in Fig. 3, the influence of dissolved CO₂ was smaller in the case of the MilliQ solutions than for natural water but still remained substantial. A purging time of 3 to 4 minutes was considered sufficient. Mean values and SD for the ≥3 min purging time range (≥1 min for K-biphtalate) are also given in Fig.3. The mean δ¹³C value (-27.58 ± 0.05‰, 1σ) found for the river fulvic acid solution along the 3 to 10 min purge time range conformed to the value given by the IHSS (-27.6‰). Assays with unacidified or acidified but unpurged fulvic acid solution, beside having a less negative mean δ¹³C value, also had a larger SD, likely due to variable levels of contamination by CO₂. Duplicate tests with nitric acid did not significantly change the mean values and SD. On the contrary, an alteration in the peak shape and more negative values were found when HCl was used, due to an incomplete oxidation of organic carbon in the reactor. The interference of halides in WCO methods has been overcome in large volume
reactor systems by adding larger quantities of persulphate.\cite{2,3} This interference seems however to be an obstacle when analysing low DOC saline waters with the LC interface used in this work. The potential effects of adding acid in samples were commented on by De Troyer et al.\cite{6} who concluded that the NPOC treatment was acceptable for HTC $\delta^{13}$C analysis. The long term storage of acidified rich DOC samples should however be avoided because of the possible flocculation of macromolecular substances. This will increase the risk of plugging the on-line filter set at the entry of the LC interface reactor and of losing a fraction of the sample DOC pool, potentially resulting in biased $\delta^{13}$C values.

**Linearity - Background and blank levels - Quantification of DOC**

Using benzoic acid solutions, Krummen et al.\cite{7} in the original publication on the LC IsoLink interface found the linearity of the system to range from 200 to 2000 ng C. Dilution series of the same solutions used above (Fig. 3) were analysed to confirm the compatibility of the method with the concentration range and the type of organic matter found in natural waters. As shown in Fig. 4, mean $\delta^{13}$C values were within about ±0.1 SD for the range 1-10 mg DOC/L (i.e. 100-1000 ng C). The mean values for the soil water sample and the stream fulvic acid were not distinguishable from Fig. 3 runs. The value calculated for K-biphtalate by this run was less negative (-26.3‰ against -26.8‰) but the mean value was still within a 0.3‰ SD.

With the water mobile phase used in this study a m/z 44 low background between 60 and 70 mV was maintained. The pulse intensity of the CO$_2$ monitor gas was set around 2 V (1750 mV in Fig. 1) by fixing gas pressure at the LC IsoLink interface entry gauge. Over the years, many factors may cause variation in the intensity of the system signal\cite{8} (e. g. new filament, source focussing, adjustment of the open split units and He flows) which can be monitored by fixing the reference gas entry pressure. Unlike HTC systems, the mobile phase and reagents continuous flow principle of the LC interface generates no instrument blank. Injection of 100 µL of degassed acidified MilliQ water gave however a blank signal around 1±0.5 Vs (equal to about 0.1 mg DOC/L), mostly attributable to the acid introduced into the samples. No blank correction of $\delta^{13}$C values was considered necessary from a concentration limit of 1 mg DOC/L. Under that limit the signal intensity (area in Vs) appears too small to give a correct calculation of $\delta^{13}$C values in accordance with linearity evaluations.

Solutions of either K-biphtalate or benzoic acid (IVA Analysentechnik, Meerbusch, Germany) were used as DOC standards for calibration with no difference in the concentration (mg C/L) versus IRMS peak area (Volt·sec) relations. Calibration may however vary over the
years by more than 50% and needs to be checked daily. DOC quantification down to 0.25 mg C/L was possible with a precision (SD) of ≤ 0.02 mg/L and an standard error of about 0.1 mg C/L (n = 10). Comparison with quantification using a high temperature catalytic total organic carbon (TOC) analyzer (Shimadzu TOC 5000A) is shown in Fig.5 for part of the whole sample set. Considering the delays between the two quantification runs and the multiplicity of subsamples, the 1 mg C/L standard error concordance indicates no systematic error in DOC concentrations obtained by LC-IRMS. A recent better-controlled interlaboratory comparison test (LC IsoLink-IRMS; ISTO versus Shimadzu TOC 5000 LGE/IPGP; Laboratoire de Géochimie des Eaux / Institut de Physique du Globe de Paris) on stream waters from the Amazonas-Rio Negro River system was conducted with only a 0.25 mg/L standard error in a 2-8 mg/L DOC range (unpublished results).

APPLICATION TO NATURAL WATER
Mapping DOC δ¹³C in soil waters at the plot scale
Results are shown in Fig. 6 and Table 1. Values fell in a narrow range, between -27.4 and -29.9‰ averaging as a whole -28.7 ± 0.6‰ (1σ, n=70). The soil water δ¹³C values were only averaged to compare to stream water values and to separate domains in the plot. Actually the spatial distribution of the values (although not large enough to be analysed statistically) does not appear to be uniform. A cluster with the more negative values is distinguishable overlapping previously defined sector (ii), where higher DOC concentrations were assumed to be linked to upper levels of the water table and an enhanced contact between water and organic top soil layers[10].

Comparison with the stream waters draining the plot
Both the δ¹³C of bulk stream DOC (-28.8 ± 0.4‰, n=3) and the XAD4 TPHacid fraction (-28.5‰), matched the plot average soil water value (-28.7 ± 0.6‰). However the more hydrophobic XAD8 HPOacid fraction (-30.0‰) was found to be 1.5‰ more negative, being therefore closer to the set of values found in the sector (ii) (green grass symbol in Fig. 6). The more negative δ¹³C signature of hydrophobic fractions had already been documented for example in forest soils by Kaiser et al.[16] who found a difference of about 3‰. This general characteristic of hydrophobic fractions was attributed to the larger contribution of compounds derived from lignin that are depleted in ¹³C.
The comparison between the TPHacid and HPOacid δ\(^{13}\)C in the stream and the spatial DOC δ\(^{13}\)C distribution in the plot suggests that the different areas in the plot may contribute differently to the stream DOC pool. Well-aerated and drained hedgerow banks may produce more hydrophilic and transphilic organic fractions (less negative δ\(^{13}\)C values) whereas more poorly drained places may supply a more hydrophobic pool (more negative values). This hypothesis agrees with the higher ionic strength and more oxic conditions prevailing in the proximity of hedgerows\[^{10}\] which are factors known to enhance flocculation and sorption of the more hydrophobic dissolved humic substances\[^{11,12}\] and thus to influence DOC δ\(^{13}\)C evolution through soil profiles\[^{15,16,24}\].

To ascertain this, analysis of the hydrophobic and hydrophilic pools of the soil waters should be carried out. The off-line extraction and concentration of organic fractions by XAD resins are however highly time- and sample-consuming while on-line LC-IRMS methods are opening up new perspectives. The application of LC-IRMS coupled size exclusion chromatography (SEC) to the XAD resin fractions analysed in this study\[^{25}\] although potentially a promising tool for discriminating dissolved substances on a molecular weight and stable carbon isotope basis, did not answer the question. The on-line analytic separation of hydrophilic and hydrophobic fractions on small volumes\[^{23}\] would be a better approach provided that it is adapted to LC-IRMS constraints. Temperature programmable LC capabilities\[^{26}\] should preferably be explored.

Conclusions and recommendations

Although the above discussion requires further work to be conclusive, the LC IsoLink interface was shown to be a useful high-sensitivity tool for the reproducible and accurate IRMS on-line determination of δ\(^{13}\)C values of DOC in fresh waters. The technique allows large sampling and a fast field survey in a comparable approach to that developed by Jochmann et al.\[^{9}\] in another domain. Analysis in triplicate takes only 10 mn and consumes small sample volumes (a few mL, 100µL single injection) of natural water just filtered and acidified. Accurate δ\(^{13}\)C determination was possible down to 1 mg DOC/L and DOC quantification down to 0.25 mg C/L with a precision (SD) of ≤ 0.02 mg/L and an standard error of about 0.1 mg C/L. Comparison with the HTC TOC analyzer did not reveal any systematic error in DOC evaluation by this WCO-based interface. Compared to other existing HTC or WCO methods, a further advantage of the LC-IRMS is that there is no need to apply blank corrections for δ\(^{13}\)C calculations. Extreme care should be taken during the purge step.
prior to injection since experience has proved that an uncontrolled He flow deficiency during manual purge was certainly the main cause of failed analysis. The use of an automatic sampler with a gas sparging option (as in most commercial TOC analyzers) would be a significant improvement. The main drawback of the WCO principle of the LC-IRMS interface would concerns the analysis of low DOC saline solutions owing to only partial oxidation of organic compounds in the on-line reactor.

Acknowledgments
Sophie Cornu (INRA) and Ary Bruand (ISTO) are warmly thanked for their help during the winter field work and Dieter Juchelka (Thermo Scientific, Bremen) for technical support. The referees are sincerely acknowledged for their valuable comments which have contributed to improving the manuscript.
References


Table 1. $\delta^{13}C$ composition relative to VPDB

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\delta^{13}C$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil Water DOC (average, n=70)</td>
<td>-28.7 ± 0.6</td>
</tr>
<tr>
<td>Soil water DOC (maximum)</td>
<td>-27.4</td>
</tr>
<tr>
<td>Soil water DOC (minimum)</td>
<td>-29.9</td>
</tr>
<tr>
<td>Bulk Stream DOC (average, n=3)</td>
<td>-28.8 ± 0.4</td>
</tr>
<tr>
<td>Bulk TPHacid (stream)</td>
<td>-28.5</td>
</tr>
<tr>
<td>Bulk HPOacid (stream)</td>
<td>-30.0</td>
</tr>
</tbody>
</table>
Figure captions

Figure 1. Triplicate DOC $\delta^{13}$C flow injection analysis (FIA). Run starts with 3 monitor gas pulses for $\delta^{13}$C calculation and ends with 2 for control. Traces of m/z 44, 45 and 46 (two later amplified) are indicated. Full loop injection 100 µL. Sample preparation: filtration, acidification, gas purge. Mobile phase: MilliQ water pH 2 with H$_3$PO$_4$, 300 µL/min; T reactor 100°C; reagents: H$_3$PO$_4$ 1.5M 50µL/min; Na$_2$S$_2$O$_8$ 0.4M 40µL/min. Triplicate analysis in 10 min.

Figure 2. Accuracy of the system. Calibration of the $\delta^{13}$C value of the CO$_2$ monitor gas over a set of reference standard compounds dissolved in water. Stability over time of the system was controlled by plotting the reference standard $\delta^{13}$C values versus measured values. The set of reference materials ranged from -10.45‰ (sucrose IAEA-CH-6) to -27.6‰ (Suwannee River fulvic acid IHSS-1S101F). Intermediate $\delta^{13}$C values correspond to sucrose IAEA-C6, -10.8‰; oxalic acid IAEA-C8, -18.3‰ and glutamic acid USGS-40, -26.39‰. Standard deviations for triplicate were less than the mark size. The difference between targeted and measured values did not exceed 0.5‰. Least-squares linear regression of the data can be used to correct measured values within a 0.3‰ difference.

Figure 3. $\delta^{13}$C values as a function of He purge time for three different solutions: Suwannee River fulvic acid IHSS-1S101F, K-biphtalate and a natural soil water sample. Symbols and error bars represent mean ± standard deviation (SD) for triplicates. The mean $\delta^{13}$C values for the ≥3 min purging time range (≥1 min for K-biphtalate) are also given.

Figure 4. Linearity. $\delta^{13}$C values against DOC concentrations for dilution series of the three different solutions as in Fig.3.

Figure 5. Comparison between DOC determinations by LC-IRMS flow injection analysis (FIA) and HTC Shimadzu TOC Analyzer. The solid line indicating the trend (and the correlation coefficient) between the two sets of data did not significantly diverge from the 1:1 line (dotted). In view of the delays between quantification runs and the multiplicity of subsamples, the ±1 mg C/L standard error (n = 63) indicates no systematic error in DOC estimation by LC-IRMS (see text).
Figure 6. Spatial distribution of soil water DOC $\delta^{13}$C in the plot. Most of the more negative values ($\leq -29.3\%o$) overlapped sector (ii) in the central zone of the plot (green grass symbol), while less negative values were generally found along hedgerows. Black contour lines refer to soil surface elevation in metre above sea-level. Abscissa and ordinate tick intervals : 10 metres.
Soil water W7: $\delta^{13}\text{C}_{\text{DOC}} = -27.99\% ± 0.02\%$

Total area = 74 Vs ± 0.3 Vs
Figure 2

\[
\begin{align*}
\delta^{13}C_{\text{measured}} & \quad \delta^{13}C_{\text{std}} \\
-30 & \quad -25 \quad -20 \quad -15 \quad -10 \\
\end{align*}
\]

- IAEA-CH-6
- IAEA-C6
- USGS-40
- IHSS-1S101F
- IAEA-C8
Figure 3

- IHSS -1S101F (5 mg C/L)
  -27.58 ± 0.05‰ (n = 6)

- K-Phtalate (6 mg C/L)
  -26.83 ± 0.06‰ (n = 4)

- Soil water J36 (DOC 6.5 mg C/L)
  -28.38 ± 0.04‰ (n = 3)
Figure 4

**IHSS-1S101F**
-27.50 ± 0.08‰ (n = 7)

**K-Phtalate**
-26.33 ± 0.13‰ (n = 7)

**Soil water J36**
-28.35 ± 0.09‰ (n = 3)
Figure 5

\[ y = 0.97x \]

\[ R^2 = 0.92 \]