한국환경분석학회지 제21권 (제4호) 229~236, 2018
J. of the Korean Society for Environmental Analysis

Inter-laboratory Comparison of Stable Carbon and Nitrogen Isotopic Composition Data Using Elemental Analyzer-isotope Ratio Mass Spectrometers

Jung-Hyun Kim[1,†], Sujin Kang[2], Yeon-Sik Bong[3], Kwangkyu Park[1], Tae-Woo Kang[4], Yong-Se Park[5], Dahae Kim[1], Seunghyun Choi[3], Young Ji Joo[1], Bohyung Choi[2], Seung-Il Nam[1], Sang-Mo Lee[5], and Kyung-Hoon Shin[2]

[1]Division of Polar Paleoenvironment, Korea Polar Research Institute (KOPRI), Incheon 21990, Korea
[2]Department of Marine Science and Convergence Technology, Hanyang University, Ansan 15588, Korea
[3]Division of Earth & Environmental Sciences, Korea Basic Science Institute (KBSI), Cheongju 28119, Korea
[4]Yeongsan River Environment Research Center, National Institute of Environmental Research, Gwangju 61011, Korea
[5]National Instrumentation Center for Environmental Management, Seoul National University, Seoul 08826, Korea

Received August 3, 2018/Revised November 5, 2018/Accepted November 27, 2018

In this study, inter-laboratory comparison was done using elemental analyzer-isotope ratio mass spectrometers (EA-IRMSs) to determine carbon and nitrogen contents as well as stable carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) of five environmental samples containing lake and marine sediments, higher plant leaves, and fish muscle, and one organic analytical standard (Protein (Casein) Standard OAS). Five national laboratories participated in this comparison study, and each laboratory analyzed all five samples and the analytical standard. Results showed that variations in total organic carbon (TOC) and total nitrogen (TN) contents as well as $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$ values among the laboratories were large compared to the analytical uncertainties. The results highlighted the inhomogeneity of the test samples and thus, the need to select suitable standard reference materials for future inter-laboratory studies. Further inter-laboratory comparison exercises could promote good measurement practices in the acquisition of stable carbon and nitrogen isotopic composition data.

Key words: Inter-laboratory comparison, Stable carbon isotope ($\delta^{13}C$), Stable nitrogen isotope ($\delta^{15}N$)

1. Introduction

The determination of stable carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) have become increasingly important in a variety of research fields, including geochemistry, hydrology, ecology, archeology, and forensic science[1-6]. An elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) has advantages of quick analysis and small sample volume. The acquisition of the EA-IRMSs has thus increased in various research institutes in South Korea. However, research using EA-IRMS is currently at a nascent stage in South Korea compared to advanced countries. Hence, it is necessary to improve the competitiveness of South Korean researchers in different research fields. There have been cases of cross-validation among analytical institutions in other countries analyzing various isotope systems in a range of materials. For instance, $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ of organic compounds, bone collagen, or materials of potential forensic interest such as packaging and pharmaceuticals[7-9] were measured in order to ascertain consistency and robustness of their stable isotope measurement results. Similar inter-laboratory comparison studies, however, are yet to be conducted in South Korea.

†To whom correspondence should be addressed.
Tel: 82-32-760-5377, Fax: 82-32-60-5397, E-mail: jhkim123@kopri.re.kr

Here, for the first time, the determination of carbon and nitrogen contents as well as stable carbon and nitrogen isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) in various environmental samples by a comparison study among research institutes in South Korea was attempted. The objective was to distribute environmental samples to the participating laboratories to allow them to assess the proficiency and accuracy of their analytical techniques. We expect that the differences among laboratories would be within the range of the analytical uncertainties reported from each laboratory, if the test samples are ideally homogenized. This inter-laboratory comparison study was a co-operative study; analysts used a method common to their own laboratories and no certification was provided. Stable carbon and nitrogen isotopes were measured in five environmental samples (lake and marine sediments, higher plant leaves, and fish muscle) and one certified organic analytical standard. They represent a wide range of environmental samples that are commonly used in the environmental sciences. In addition, easy access to these samples allowed us to obtain enough volumes for distributing sufficient subsamples to the participating laboratories.

2. Experimental Methods

2.1. Participating laboratories

Firstly, the South Korean research institutes with EA-IRMSs were surveyed and asked to participate in this inter-laboratory comparison exercise. According to the survey, a total of 24 South Korean institutes had an EA-IRMS at the time of the survey and seven institutes among them showed interest in participating. However, two institutes could not join this comparison exercise due to their analysis schedule. Participating laboratories were identified by letter only and their results were shown in an arbitrary order.

2.2. Sample preparations

The samples for distribution were prepared at Hanyang University. Five different environmental samples were used: lake and marine sediments, higher plant leaves, and fish muscle (Fig. 1). One lake sediment sample was collected from Lake Shihwa in May 2013 using a grab sampler. Two marine sediment samples were collected from the temperate and polar regions, i.e. from the East Sea (ES14-BC01; 37°12.05'N, 130°25.09'E; 2160 m water depth) in May 2014 and the Chukchi Sea (ARA01B/04MUC-01; 73°44.10'N, 167°0.25'E; 43 m water depth) in August 2010 with R/V ARAON using a box corer and a multi-corer, respectively. Leaves of pine trees were gathered at Hanyang University in October 2016. One *Mugil cephalus* was captured in the estuary of the Geum River flowing into the Yellow Sea in May 2016. All samples were stored at –20°C, freeze-dried, and then ground to a fine powder using an agate mortar and pestle. Each of the powdered samples were collected in a glass jar and rigorously mixed manually several times and then using a multi funnel shaker (MMV-1000W, EYELA, Japan). This mixing procedure was repeated several times over several days. In addition, one organic analytical standard (Protein (Casein) Standard OAS) was purchased from Elemental Microanalysis Ltd (Devon, UK) (Cat no. B2155 - Certificate no.114859). Two aliquots (one for raw sample measurements and one for decalcified sample measurements) of each type of sample, approximately 2 g for each aliquot, were transferred into clean, labelled vials with no further processing and sent to each of the participating laboratories.

2.3. Grain size analysis

Seven aliquots of each environmental sample (a total of 35) were randomly subsampled and used for grain size analysis, which was performed at the Korea Basic Science Institute (KBSI) in Jeonju to test the physical homogeneity of the environmental samples. All the samples were analyzed with the dynamic light scattering (DLS) method using a particle size analyzer (Microtrac UPA150, Microtrac

Inc., USA)[10]. In addition, five aliquots each of the Lake Shihwa and East Sea sediments were randomly subsampled and analyzed using a SediGraph III particle size analyzer (Micromeritics Inc., USA)[11].

2.4. EA-IRMS analysis

For the carbon and nitrogen contents and the stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope measurements of both non-decalcified and decalcified materials, the laboratories were requested to process the samples in their usual way. The analyses were carried out using different brands of EA-IRMSs. Participating laboratories were requested to perform the analysis at least twice with each aliquot of the samples. The final total organic carbon (TOC) value was calculated using the following equation: TOC = (100 – (8.333 × TC)) / ((100/TOC') – 8.333)[12]. Note that TOC' denotes carbon contents obtained from the decalcified samples, while TC represents the total carbon content of the non-decalcified samples. Note that $\delta^{13}C$ values of decalcified samples (i.e. $\delta^{13}C_{TOC}$) were reported in this study. In contrast, total nitrogen (TN) contents and $\delta^{15}N_{TN}$ values obtained from the non-decalcified samples were used in this study. Each laboratory recorded their analytical results in a form that was supplied with the samples for keeping track of information on analytical instrumentation, decalcification processes, and calibration standards with certificated values.

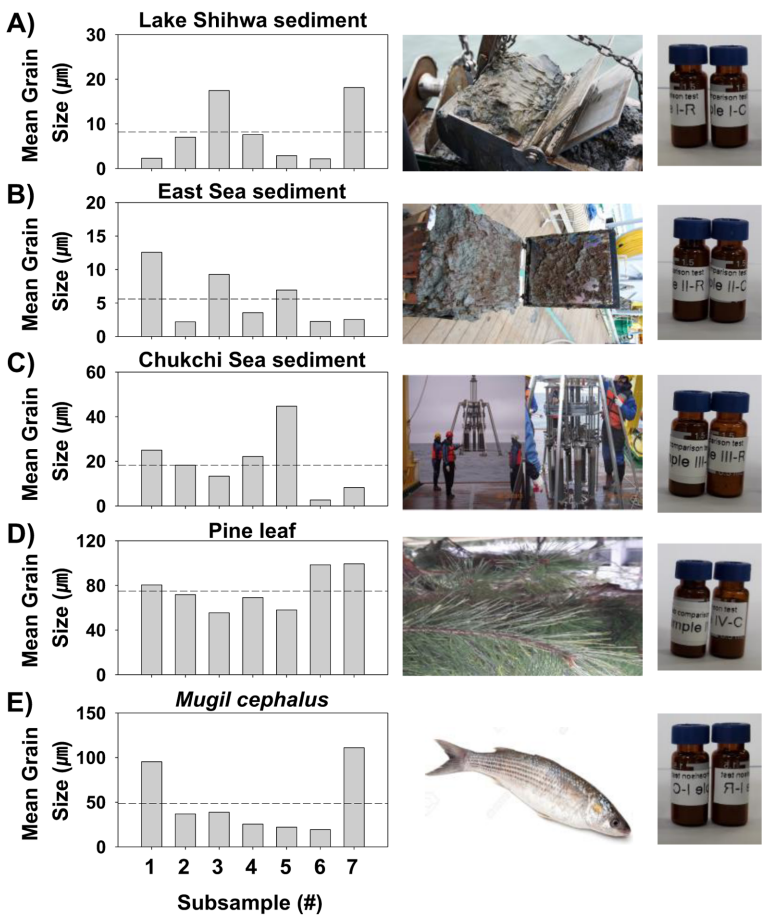

Fig. 1. Results of the grain size analyses: A) Lake Shihwa sediment, B) East Sea sediment, C) Chukchi Sea sediment, D) pine leaf, and E) *Mugil cephalus*. The dotted horizontal lines show the mean value of all the results.

3. Results and Discussions

The sample homogeneity was estimated using the grain size distribution of the environmental samples (Fig. 1). The mean grain size of the seven measurements obtained by the DLS method were 8.2±6.4 mm for the Lake Shihwa sediment, 5.6±3.8 mm for the East Sea sediment, 19.2±12.6 mm for the Chukchi Sea sediment, 76.1±16.4 mm for the pine leaf, and 49.834.7 mm for *Mugil cephalus*. The grain size variations were large, suggesting that the samples were inhomogeneous. To verify the DLS results, the grain sizes of two sediment samples were analyzed using a different method, i.e., the SediGraph method. The SediGraph results showed a much smaller variation, with mean values of 2.3±0.3 mm for the Lake Shihwa sediment and 10.0±0.4 mm for the East Sea sediment. In general, the DLS method is more suitable for samples with low concentrations and limited quantities, while the SediGraph method required much larger sample amount (>0.5 g)[13]. Accordingly, the larger grain size variations obtained by the DLS method might be due to the smaller sample size used for the grain size analysis. Nonetheless, it is evident that the environmental samples used for the inter-labor-

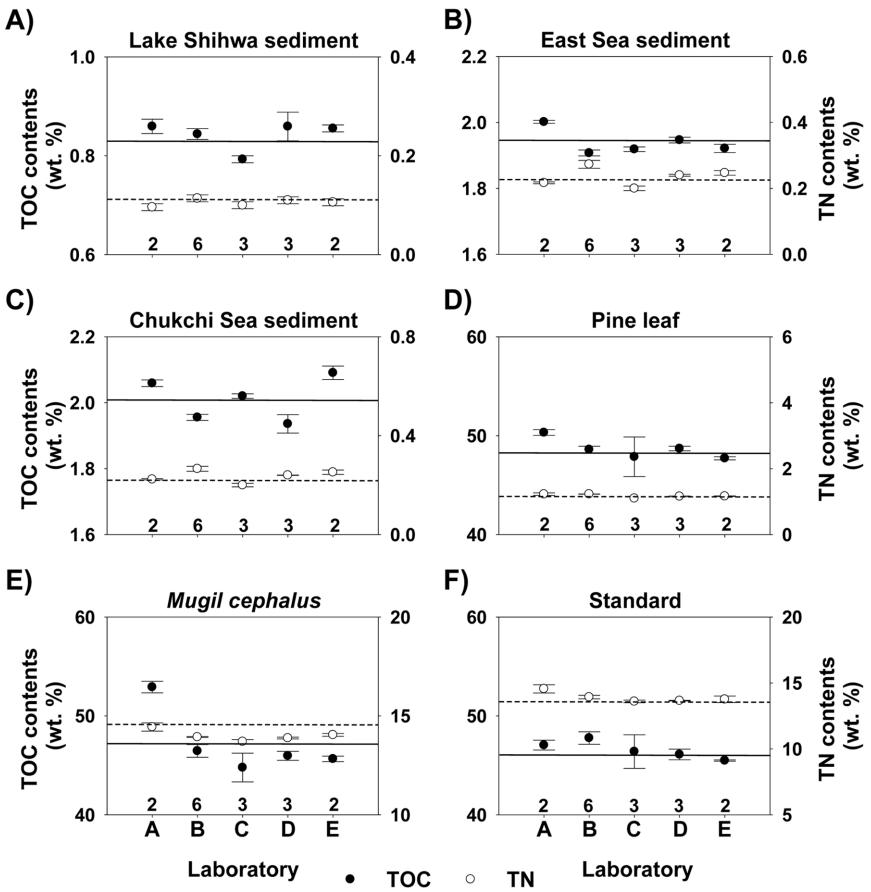

Fig. 2. Distribution of the initial TOC and TN contents as per each laboratory: A) Lake Shihwa sediment, B) East Sea sediment, C) Chukchi Sea sediment, D) pine leaf, E) *Mugil cephalus*, and F) organic analytical standard. Filled and open circles represent the individual analyses for TOC and TN, respectively. The solid and dotted horizontal lines show the mean values of all the participating laboratories for TOC and TN, respectively. Error bars represent ±1σ standard deviation of the individual laboratory data. Numbers denote the number of measurements conducted in each laboratory.

atory comparison study were inhomogeneous.

The mean TOC contents for all participating laboratories were 0.84±0.03 wt.% for the Lake Shihwa sediment, 1.94±0.03 wt.% for the East Sea sediment, 2.01±0.06 wt.% for the Chukchi Sea sediment, 48.64±0.93 wt.% for the pine leaf, 47.15±2.93 wt.% for *Mugil cephalus,* and 46.56±0.78 wt.% for the organic analytical standard (Fig. 2). The mean TN contents for all participating laboratories were 0.11±0.01 wt.% for the Lake Shihwa sediment, 0.24±0.03 wt.% for the East Sea sediment, 0.24±0.02 wt.% for the Chukchi Sea sediment, 1.18±0.05 wt.% for the pine leaf, 14.00±0.25 wt.% for *Mugil cephalus* and 13.90±0.34 wt.% for the organic analytical standard (Fig. 2). Across all the participating laboratories, $\delta^{13}C_{TOC}$ values averaged -22.45 ±5.03‰ for the Lake Shihwa sediment, -21.30±3.09‰ for the East Sea sediment, -21.84±3.83‰ for the Chukchi Sea sediment, -30.70±0.85‰ for the pine leaf, -17.00±0.20‰ for *Mugil cephalus* and -27.12±0.36‰ for the organic analytical standard (Fig. 3). The $\delta^{15}N_{TN}$ for all participating laboratories averaged 6.39±1.62‰ for the Lake Shihwa sediment, 5.66±1.05‰ for the East Sea sediment, 8.42±1.16‰ for the Chukchi Sea sediment, -3.11 ±0.49‰ for the pine leaf, 12.71±0.39‰ for *Mugil*

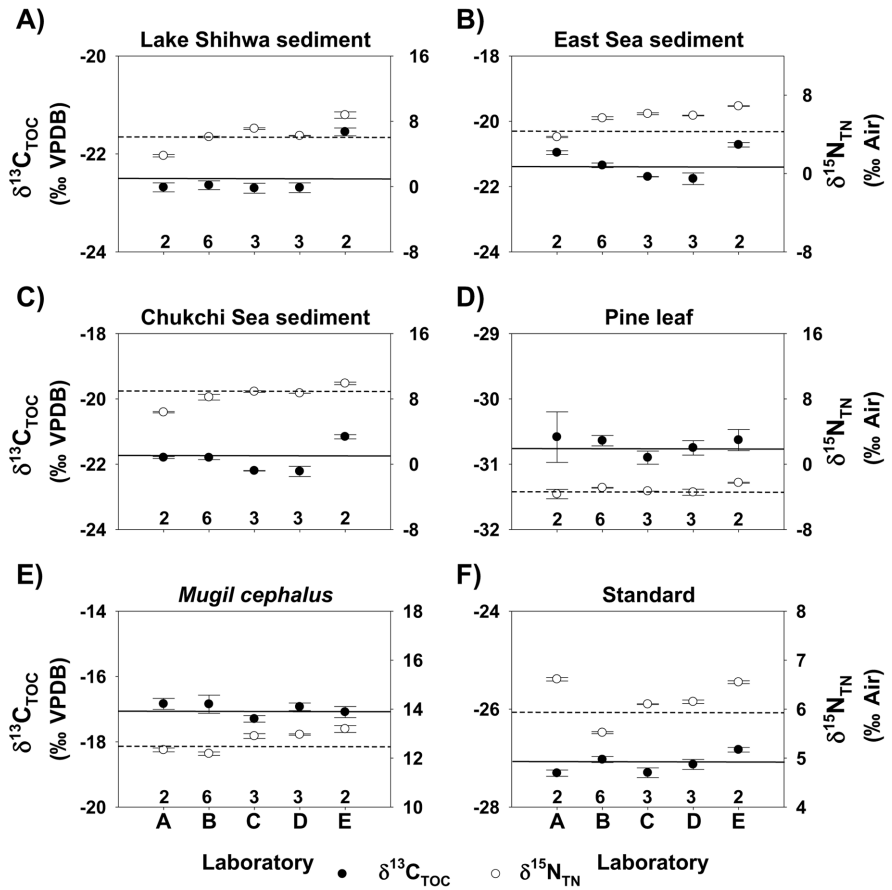

Fig. 3. Distribution of the initial $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$ values by each laboratory: A) Lake Shihwa sediment, B) East Sea sediment, C) Chukchi Sea sediment, D) pine leaf, E) *Mugil cephalus*, and F) organic analytical standard. Filled and open circles represent the individual analyses for $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$, respectively. The solid and dotted horizontal lines show the mean values of all the participating laboratories for $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$, respectively. The error bars show ±1σ standard deviation of each participating laboratory. The numbers denote the number of measurements conducted in each laboratory.

cephalus and 6.19±0.39‰ for the organic analytical standard (Fig. 3).

The difference between the mean values of all the participating laboratories and the mean values of data reported by an individual laboratory was calculated (Fig. 4). In the case of the organic analytical standard, the difference between the mean values of data reported by the individual laboratory and the certified values provided (Elemental Microanalysis Ltd, Devon, UK) was calculated. For the TOC and TN contents, the differences were in the range of 0.07 (-0.02 to 0.05) and 0.02 (-0.01 to 0.01) wt.%, respectively, for the Lake Shihwa sediment, 0.09 (-0.06 to 0.03) and 0.07 (-0.04 to 0.04) wt.%, respectively, for the East Sea sediment, 0.15 (-0.08 to 0.08) and 0.07 (-0.03 to 0.04) wt.%, respectively, for the Chukchi Sea sediment, 2.60 (-1.68 to 0.92) and 0.13 (-0.05 to 0.08) wt.%, respectively, for the pine leaf, and 8.13 (-5.76 to 2.37) and 0.74 (-0.44 to 0.30) wt.%, respectively, for *Mugil cephalus*. The certified carbon and nitrogen values of the organic analytical standard were 46.5±0.78 and 13.32±0.40 wt.%, respectively. The differences were between -1.27 and 1.01 wt.% for the TOC content, and between -1.23 and -0.28 wt.% for the TN content. In general, the TOC and TN differences of the sediment samples were much smaller than those of the plant and fish samples. Moreover, the differences in the TN contents were smaller than those in the TOC contents. This could be due to,

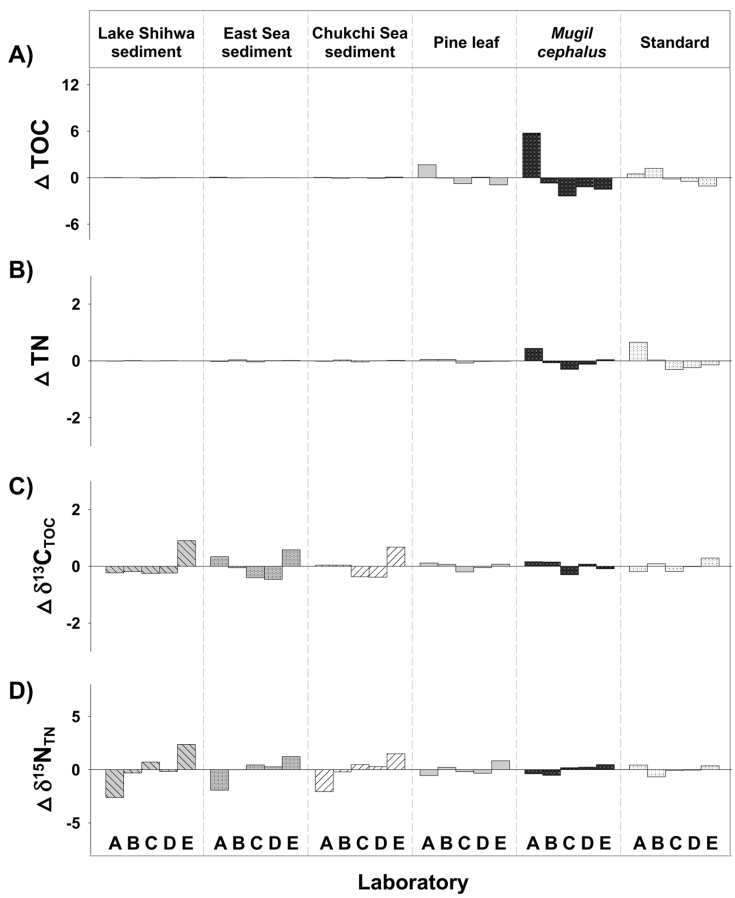

Fig. 4. Difference between the mean values of all participating laboratories and the mean values of data reported by an individual laboratory or the certified values (for the analytical standard): A) ΔTOC, B) ΔTN, C) $\Delta\delta^{13}C_{TOC}$, and D) $\Delta\delta^{15}N_{TN}$.

Table 1. Summary of the decalcification method used in the laboratories participated in this study

Lab code	Decalcification method
A	1) Put a suitable amount of samples into the 50 ml beaker and add the 10~15 ml of 10 % HCl. 2) Keep it in room temperature for 24 hr. 3) Add the distilled water after removal of the supernatant and stay it during the 24 hr for the settling of the samples. 4) Repeat the step 3) for 4 times and remove the supernatant. 5) Check the pH using a litmus paper and dry remaining water in the oven. 6) Grind and homogenize the sample.
B	1) Add a suitable amount of 1 N HCl to a small amount of samples. 2) React it during the 12 hr. 3) Neutralize the samples using ultrapure water for 3 times. 4) Remove the water using a freeze-dryer.
C	1) Add the 10 ml of 8 % HCl to the sample and shake it. 2) Remove the supernatant by centrifugation at 3000 rpm for 10 minutes. 3) Add deionized water and shake it. 4) Remove the supernatant by centrifugation at 3000 rpm for 10 minutes. 5) Repeat the step 3) to 4) for 2 times. 6) Dry the samples in the 50°C oven.
D	1) Put a small amount of samples in a conical tube and add the 1 M HCl. 2) Shake it to react overnight. 3) Remove the supernatant by centrifugation. 4) Add ultrapure water and shake it for neutralization. 5) Remove the supernatant by centrifugation. 6) Repeat the step 4) to 5) again and remove the water using a freeze-dryer.
E	Not applied.

at least partly, the different decalcification processes applied in each participating laboratory, considering that the TN contents were obtained from the non-decalcified samples (Table 1).

For $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$, the differences were in the range of 1.15 (-0.90 to 0.25) and 4.98 (-2.37 to 2.61)‰, respectively, for the Lake Shihwa sediment, 1.04 (-0.58 to 0.46) and 3.15 (-1.22 to 1.93)‰, respectively, for the East Sea sediment, 1.06 (-0.68 to 0.38) and 3.53 (-1.48 to 2.05)‰, respectively, for the Chukchi Sea sediment, 0.31 (-0.12 to 0.20) and 1.39 (-0.84 to 0.55)‰, respectively, for the pine leaf, and 0.46 (-5.76 to 2.37) and 0.74 (-0.16 to 0.30)‰, respectively, for *Mugil cephalus*. The certified stable carbon and nitrogen isotope values of the organic analytical standard were -26.98±0.13 and 5.94±0.08‰, respectively (Elemental Microanalysis Ltd, Devon, UK). The differences were between -0.15 and 0.33‰ for $\delta^{13}C_{TOC}$ and between -0.67 and 0.42‰ for $\delta^{15}N_{TN}$. In contrast to the TOC and TN contents, the differences in $\delta^{15}N_{TN}$ were larger than those in $\delta^{13}C_{TOC}$. Abundant nitrogen in ambient air, which can be easily introduced into the IRMS system via tiny leaks undetected during the routine analysis procedure, in combination with the low nitrogen levels in the analyzed samples can account for the large discrepancies. Furthermore, the differences in $\delta^{13}C_{TOC}$ among laboratories were large compared to the analytical uncertainties reported from each laboratory. The results of this study suggest that, in general, the differences between the $\delta^{13}C_{TOC}$ data derived from different laboratories could, to some extent, be due to the differences in sample pretreatments (Table 1).

4. Conclusions

This paper presents the first inter-laboratory comparison of stable carbon and nitrogen content and isotope measurements in South Korea. In this comparison, aliquots of five environmental samples and

one organic analytical standard were prepared and distributed to five South Korean laboratories. The samples provided were further processed with each laboratory's standard method and analyzed. The results showed that inter-laboratory variations in TOC and TN contents, as well as $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$ values, were large compared to the analytical uncertainties reported by each participating laboratory. The variations could have resulted from the inhomogeneity of the environmental samples. In addition, the variations could be due to the differences in sample pretreatment. For better comparing the differences between the results obtained by the laboratories, further study to analyze diverse organic analytical standard materials and a much larger number of participating laboratories are necessary. Mutual verification of the analytical technology among South Korean research institutes can improve the reliability of analytical results. Hence, greater participation by a wider range of South Korean research institutions in inter-laboratory comparisons in future is encouraged.

Acknowledgements

The authors would like to thank to Dong-Hun Lee and Jong-Ku Gal for their sampling and analytical support at HYU and Heung-Soo Moon and Young-Jin Joe at KOPRI for their assistance for the grain size analysis. This study was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Science and ICT (MSIT) (NRF-2016R1A2B3015388, PN18100), and "Development of practical technique to establish fisheries forensic" funded by the Ministry of Oceans and Fisheries, Korea.

References

1. M. J. DeNiro and S. Epstein, "Influence of diet on the distribution of nitrogen isotopes in animals", *Geochimica et Cosmochimica Acta*, **1981**, 42, 495-506.
2. M. J. Schoeninger, M. J. DeNiro and H. Tauber, "$^{15}N/^{14}N$ ratios of bone collagen reflect marine and terrestrial components of prehistoric diets", *Science*, **1983**, 220, 1381-1383.
3. S. H. Ambrose, B. M. Butler, D. B. Hanson, R. L. Hunter-Anderson and H. W. Kruegers, "Stable isotopic analysis of human diet in the Marianas Archipelago, Western Pacific", *American Journal of Physical Anthropology*, **1997**, 104, 343–361.
4. M. J. Perkins, R. A. McDonald, F. J. F. van S. D. Veen, Kelly, G. Rees and S. Bearhop, "Application of nitrogen and carbon stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) to quantify food chain length and trophic structure", *PLoS ONE*, **2014**. 9, e93281.
5. F. Hyodo, "Use of stable carbon and nitrogen isotopes in insect trophic ecology", *Entomological Science*, **2015**, 18, 295-312.
6. B. Wang, Y. Wul and D. Chen, "Stable isotopes of carbon and nitrogen help to predict the belowground communities at a regional scale", *Scientific Reports*, **2017**, 7, DOI:10.1038/s41598-017-07517-w.
7. J. F. Carter, J. C. Hill, S. Doyle and C. Lock, "Results of four inter-laboratory comparisons provided by the Forensic Isotope Ratio Mass Spectrometry (FIRMS) network", *Science and Justice*, **2009**, 49, 127-137.
8. Y. Suzuki, Y. Chikaraishi, K. Yamada and N. Yoshida, "Interlaboratory comparison of carbon, nitrogen, and oxygen isotope ratios in organic chemicals using elemental analyzer-isotope ratio mass spectrometer", *Bunseki Kagaku*, **2012**, 61, 805-810.
9. W. J. Pestle, B. E. Crowley and M. T. Weirauch, "Quantifying inter-laboratory variability in stable isotope analysis of ancient skeletal remains", *PLoS ONE*, **2014**, 9, e102844.
10. A. Cadene, S. Durand-Vidal, P. Turq and J. Brendle, "Study of individual Namontmorillonite particles size, morphology, and apparent charge", *Journal of Colloid and Interface Science*, **2005**, 285, 719-730.
11. Micromeritics, "Instruction Manual: SediGraph Particle Size Analyser", **1978**, p. 122.
12. R. Stein, H. Grobe and M. Wahsner, "Organic carbon, carbonate, and clay mineral distributions in eastern central Arctic Ocean surface sediments", *Marine Geology*, **1994**, 119, 269-285.
13. N. H. Welch, P. B. Allen and D. J. Galindo, "Particle-size analysis by pipette and sedigraph", *Journal of Environmental Quality*, **1979**, 8, 543-546.