

Differentiation analysis of boron isotopic fractionation in different forms within plant organ samples

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ABSTRACT

As a critical micronutrient, boron (B) plays an important role in plant growth and embryonic development. To further understand the effects of B uptake, transportation and isotopic fractionation, the contents and isotopic compositions of hydro-soluble B in the sap and structural B fixed in the cell within individual plant tissues were investigated. The B isotope ratio was determined by multi-collector inductively coupled plasma mass spectrometry. The $\delta^{11}\text{B}$ values in hydro-soluble and structural B in the investigated plant samples ranged from -1.57‰ to $+11.30\text{‰}$ and from $+6.57\text{‰}$ to $+16.64\text{‰}$, respectively. Different fractionation factors of the B isotopes, in the range of 0.9954–1.0150, were observed in these samples, indicating that in most plant tissues, the heavy isotope (^{11}B) was preferentially enriched in structural B, which was fixed into the cell. However, there was a reversal in the fractionation of B isotopic compositions in the fruit samples compared with the other plant tissue samples. It is more powerful to examine the molecular mechanisms of B transport, uptake and utilization than the use of limited plant organ samples containing a mixture of hydro-soluble and structural B within different intra-plant compartments and in inter-plant interactions. These isotopic shifts, which may be used as important isotopic indicators, contribute to the surface processes interactions in the plant–soil system and the knowledge of the molecular mechanisms of B in the uptake and absorption by different plant species in nature.

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1. Introduction

Boron (B) and its isotopes (^{10}B and ^{11}B), which have relatively greater isotopic mass difference in natural samples (-70‰ and $+75\text{‰}$, respectively) (Xiao et al., 2013, 2014), have been used as powerful indicators to reconstruct pH values of the ancient seas (Farmer et al., 2015; He et al., 2013a; Nir et al., 2015; Xiao et al., 2012), trace water pollution (Meredith et al., 2013; Nisi et al., 2014), discriminate depositional environments (Vengosh et al., 1995; Xiao et al., 2013), and identify the provenance of agricultural products (Chang et al., 2015; Oda et al., 2001; Serra et al., 2005). The recent use of B isotopes in biological systems has focused on establishing the provenances of plants and foods (Chang et al., 2015; He et al.,

2013b; Oda et al., 2001; Wieser et al., 2001), using ^{10}B as an isotopic spike for plant growth (Vanderpool and Johnson, 1992), and interpreting the mechanism of B isotopic fractionations (Rosner et al., 2011).

The $\delta^{11}\text{B}$ values in various commercial products from different regions reported by Vanderpool and Johnson (1992) range from -7.5‰ to $+29.3\text{‰}$, indicating that B isotopes fractionate between plant tissues and the nutrient solutions. Rosner et al. (2011) developed a method for B separation using wet chemical digestion combined with chromatography, with the observed $\delta^{11}\text{B}$ values in crops ranging from 0‰ to $+34\text{‰}$. This suggested regionally varying contributions from the natural background and anthropogenic activity. Xu et al. (2015) described that $\delta^{11}\text{B}$ values ranged from -19.45‰ to $+28.13\text{‰}$ in different plant tissues from the Qinghai–Tibetan Plateau and Shandong areas. The $\delta^{11}\text{B}$ values in various compartments of bell pepper, which ranged from -11.0‰ to $+16\text{‰}$, increased with the plant height, which means the young plant parts became enriched with the ^{11}B as the plant grew (Geilert et al., 2016). However, in hydroponic plant growth experiments

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with ^{10}B isotopic spikes in the artificial culture solutions, wheat and corn were preferentially enriched with ^{10}B , while varying fractionations of the B isotope occurred in broccoli (Marentes et al., 1997). This indicated that the uptake and utilization of B by different intra-plant compartments or inter-plant interactions, B isotopes were preferentially used by different plant species, which resulted in the enrichment of the heavy or light isotope in tissues having different fractionation trends. In general, during the uptake of B, featured isotope signatures are caused by different absorption mechanisms for B isotopes or the transport of B. However, these observed isotopic differences among plant tissues or inter-plant interactions include all the variations of the B isotope compositions in some tissues or the whole plant. In fact, once B, in the form of $\text{B}(\text{OH})_3$, which was absorbed by the plant roots, was transferred to other tissues, some was fixed in the structure of the cell wall (Park and Schlesinger, 2002), which is, therefore, not transported nor does it participate in the exchange cycle within the plant (Blevins and Lukaszewski, 1998; O'Neill et al., 2004; Shaaban, 2010). The residual B unabsorbed in the sap, which was not initially utilized by the plant, is transported to other tissues by nutritional transporters for further utilization. In the foregoing reports, the mixture of the two forms of B was used for testing. Thus, the fractions of B isotopes that were utilized could not be examined within a plant. What's more, if the different forms of B were investigated separately, more details of the mechanisms of uptake, transportation could be obtained and the isotope fractionation could be interpreted in deep.

Based on the previous studies that investigated B isotope compositions in five different plant species in Qinghai-Tibet Plateau and Shandong areas (Xu et al., 2015), the objective of this study was to investigate the variation in the mass concentrations and isotopic compositions of hydro-soluble and structural B within plant samples. The B isotopic compositions were measured by multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS). The fractionation factors (α) of the B isotopes were investigated and discussed.

2. Results and discussion

2.1. Variation in mass concentrations of hydro-soluble and structural B in plant samples

Several samples, including the roots, leaves and stems of *Weigela florida* cv. Red Prince (A, B, and C), roots, leaves, and stems of *Swertia mussotii* Franch. (D, E, and F), flowers and stems of *Cynomorium songaricum* Rupr. (G and H), *Pyrus pyrifolia* var. *cultu* (Makino) Nakai (I), *Citrus maxima* (Burm) Merr. (J), and *Malus pumila* Mill. (K), were selected for trials to verify the variations in mass concentrations and isotopic compositions of hydro-soluble and structural B. The mass concentrations in hydro-soluble B and structural B of these samples, which are shown in Fig. 1, ranged from 13.69 to 90.91 mg kg^{-1} and from 27.21 to 67.06 mg kg^{-1} dried weight (Table 1), respectively. The plant organ samples, excluding those of the fruits, had lower amounts of hydro-soluble B than structural B (Fig. 1; left ellipse); however, in the three fruits, the amounts of hydro-soluble B were greater than those of structural B (Fig. 1; right ellipse). In plant growth, some of the hydro-soluble B was utilized by the organs to form the cell wall, which led to the accumulation of structural B in the homologous organs, which lead to the amount of hydro-soluble B being lower than of structural B in the same compartment. According to the determination, the three fruits are over 80% water. Previous reports (Blevins and Lukaszewski, 1998; Loomis and Durst, 1992) have shown that B is a chemotactic agent for propagative organ growth through reproductive tissues and that plenty of B are required for the

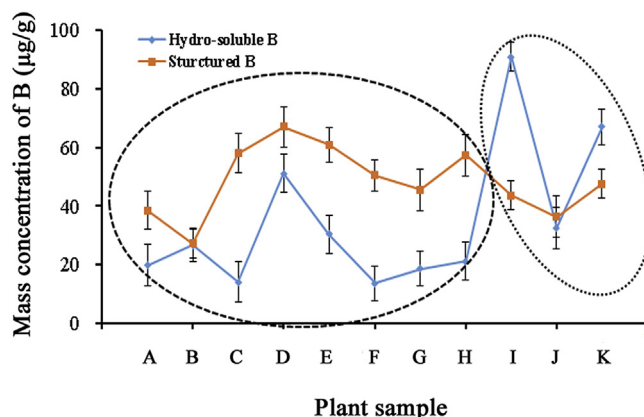


Fig. 1. Variations in mass concentrations of hydro-soluble and structural B in plant samples. A: root of *W. florida*, B: leaf of *W. florida*, C: stem of *W. florida*, D: root of *S. mussotii*, E: leaf of *S. mussotii*, F: stem of *S. mussotii*, G: flower of *C. songaricum*, H: stem of *C. songaricum*, I: *P. pyrifolia* var. *cultu*, J: *C. maxima*, K: *M. pumila*.

reproductive growth. In the sap, various *cis*-polyols, such as sorbitol, fructose, glucose, and saccharose, are utilized by combining with the hydroxyls of $\text{B}(\text{OH})_3$ and borate, which transfer B to the fruit part (Huang et al., 2014). This may be the reason for greater levels of hydro-soluble B compared with structural B in fruits.

2.2. Variation in $\delta^{11}\text{B}$ values of hydro-soluble and structural B in plant samples

The isotopic compositions of hydro-soluble and structural B in these plant organ samples were determined by MC-ICP-MS and are listed in Table 2 and shown in Fig. 2. The $\delta^{11}\text{B}$ values in hydro-soluble and structural B ranged from -1.57‰ to $+11.30\text{‰}$ ($\Delta\delta^{11}\text{B} = 12.87\text{‰}$) and from 6.57‰ to 16.64‰ ($\Delta\delta^{11}\text{B} = 10.07\text{‰}$),

Table 1
Contents of hydro-soluble B and structural B in plant organs (number of measurements, $n = 3$).

Plant	Organ	Mass concentration (mg/kg)	Added amount (μg)	RSD (%)	Recovery (%)
<i>W. florida</i>	Root	19.83 ^a	0.1	4.5	98.7
		38.44 ^b	0.1	3.6	103.1
		26.73 ^a	0.1	5.6	98.8
	Leaf	27.21 ^b	0.1	3.2	100.9
		14.05 ^a	0.1	2.8	97.7
		58.07 ^b	0.1	3.4	98.1
<i>S. mussotii</i>	Root	51.18 ^a	0.1	4.1	104.7
		67.06 ^b	0.1	3.7	104.3
		30.36 ^a	0.1	4.9	97.7
	Leaf	60.89 ^b	0.1	5.2	96.1
		13.69 ^a	0.1	3.8	102.4
		50.41 ^b	0.1	5.3	101.4
<i>C. songaricum</i>	Stem	21.08 ^a	0.1	3.4	103.5
		57.32 ^b	0.1	5.3	97.6
		18.69 ^a	0.1	2.7	102.9
	Flower	45.54 ^b	0.1	3.1	100.1
		26.43 ^a	0.1	3.3	102.6
		52.37 ^b	0.1	2.8	99.3
<i>P. pyrifolia</i> var. <i>cultu</i>	Stem	90.91 ^a	0.1	3.5	103.7
		43.58 ^b	0.1	3.4	102.3
<i>C. maxima</i>	Stem	32.62 ^a	0.1	4.3	96.5
		36.37 ^b	0.1	4.0	98.4
<i>M. pumila</i>	Stem	67.00 ^a	0.1	3.3	99.4
		47.62 ^b	0.1	4.7	103.2

^a Hydro-soluble B.

^b Structural B.

Table 2
Boron isotopic composition in plant samples.

Plant	Organ	B style	$\delta^{11}\text{B}$ (‰)	2SD(‰)
<i>W. florida</i>	Root	Hydro-soluble B	-1.57	0.09
		Structural B	10.67	0.10
	Leaf	Hydro-soluble B	4.48	0.12
<i>S. musotii</i>	Stem	Structural B	11.12	0.11
		Hydro-soluble B	1.59	0.07
	Root	Structural B	15.22	0.10
		Hydro-soluble B	10.35	0.08
	Leaf	Structural B	5.68	0.05
		Hydro-soluble B	3.97	0.09
<i>C. songaricum</i>	Stem	Structural B	12.52	0.05
		Hydro-soluble B	6.15	0.08
	Flower	Structural B	7.43	0.06
<i>H. elliptica</i>	Stem	Hydro-soluble B	5.78	0.07
		Structural B	9.32	0.06
<i>P. pyrifolia</i> var. <i>culta</i>	Stem	Hydro-soluble B	4.03	0.08
		Structural B	9.19	0.05
<i>C. maxima</i>	Stem	Hydro-soluble B	1.60	0.11
		Structural B	16.64	0.06
<i>M. pumila</i>	Stem	Hydro-soluble B	9.08	0.11
		Structural B	6.57	0.06
<i>C. maxima</i>	Stem	Hydro-soluble B	7.14	0.07
		Structural B	7.32	0.06
<i>M. pumila</i>	Stem	Hydro-soluble B	11.03	0.08
		Structural B	7.51	0.06

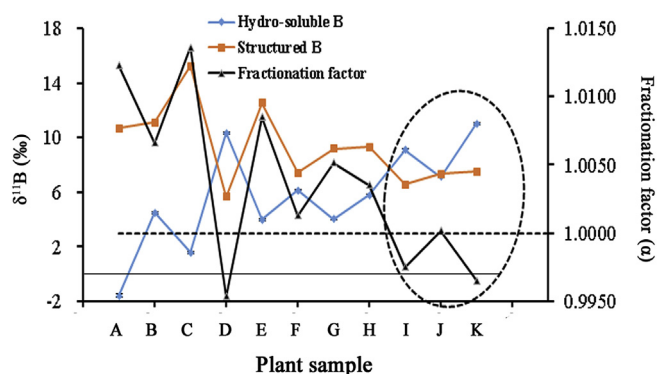


Fig. 2. Variation in the $\delta^{11}\text{B}$ of hydro-soluble and structural B and fractionation factors in selected plant samples. A: root of *W. florida*, B: leaf of *W. florida*, C: stem of *W. florida*, D: root of *S. musotii*, E: leaf of *S. musotii*, F: stem of *S. musotii*, G: flower of *C. songaricum*, H: stem of *C. songaricum*, I: *P. pyrifolia* var. *culta*, J: *C. maxima*, K: *M. pumila*.

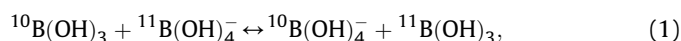
respectively. In the plant samples, the $\delta^{11}\text{B}$ values in structural B were greater than those in hydro-soluble B, except for Sample D. This indicated that in these samples, ^{11}B preferentially entered into and was fixed in the cell wall in its structural form within plant. Thus, greater amount of ^{10}B left in the sap was transported to the other organs for further use. In Sample D (root of *S. musotii*), more ^{11}B was left in the sap of the root, and more ^{10}B remained in the structural form, which was different from in the leaf (E) and stem (F) samples.

In the three fruits samples (I, J and K), the $\delta^{11}\text{B}$ values in structural B were lower than those in hydro-soluble B, which represented a reversal in the fractionation of B isotope compared with the plant samples mentioned above. This suggested that more ^{10}B was enriched in structural B by fruit, but plenty of ^{11}B remained in the sap.

2.3. Fractionation of B isotopic compositions in the two forms in plant samples

According to the report of Kakihana et al. (1977), the B isotope

reaction is not balanced, as expressed in Eq. (1), as following:



In which ^{10}B is enriched in $\text{B}(\text{OH})_4^-$ in the sedimentary phase preferentially, leaving more ^{11}B in the aqueous phase. Thus, the existing B form and its isotopic composition were dependent on the pH and the amount of B in the aqueous solution. The pH levels of the sap solution in these samples were determined and listed in Table 3. The pH values of these plant samples ranging from 3.94 to 5.83, which is less than 7, meaning that hydro-soluble B almost in the form of $\text{B}(\text{OH})_3$ was enriched with ^{11}B in the sap solution of these plant samples for absorption, transportation and uptake by the plant.

B, as a mineral micronutrient, is absorbed by higher plants through the root, and transported from soil to root, to stem, to leaf, and to flower. In these process, two B isotopic fractionation events are apparent within plant, one is B transportation between root, stem, leaf, and flower in the vertical direction, the other is the transmembrane transport of B horizontally in the cell. B isotopic fractionation occurs in different compartments within plant samples, which are located between the neighboring tissues, such as at the interface of root and stem, from stem to branch and leaf, in the interfaces of leaves, flowers and seeds (Geilert et al., 2016; Xu et al., 2015). Thus, the given organ can be considered as the recipient of nutrients from upstream organs in the transport sequence, and also a reservoir of nutrients for distribution to downstream organs. In the organ samples of *W. florida* (A, B, and C) and *S. musotii* (D, E and F) in Fig. 2, the lowest $\delta^{11}\text{B}$ values in the hydro-soluble and structural forms all occurred in the stem compartments of the two plants. Eq. (2), as follows, is based on these results.

$$\delta^{11}\text{B}_{\text{structural B-stem}} + \delta^{11}\text{B}_{\text{structural B-flower}} + \delta^{11}\text{B}_{\text{hydro-soluble B-stem}} + \delta^{11}\text{B}_{\text{hydro-soluble B-flower}} > \delta^{11}\text{B}_{\text{structural B-root}} + \delta^{11}\text{B}_{\text{hydro-soluble B-root}}, \quad (2)$$

which means that heavy B isotope is preferentially enriched in the young growth points of the tissues aboveground. Thus, the results in Fig. 2 demonstrated that in the downstream transportation of hydro-soluble B in the xylem from root to the aboveground organs, much more heavy B isotope was taken up and transported to the higher part of plant by the nutrient transporters.

Based on Eq. (3), as follows,

$$\delta^{11}\text{B}_{\text{structural B}} > \delta^{11}\text{B}_{\text{hydro-soluble B}}, \quad (3)$$

more ^{11}B in the hydro-soluble B, as seen in *W. florida* (A, B, and C), *S. musotii* (E and F), and *C. songaricum* (G and H) shown in Fig. 2, transported to the aboveground tissues were used to form the cell

Table 3
pH values of the sap solution in plant organs (number of measurements, n = 3).

Plant	Organ	pH	Deviation error
<i>W. florida</i>	Root	5.76	0.06
	Leaf	4.95	0.04
	Stem	5.14	0.08
<i>S. musotii</i>	Root	5.08	0.07
	Leaf	4.82	0.05
	Stem	4.75	0.06
<i>C. songaricum</i>	Stem	5.72	0.04
	Flower	5.83	0.07
<i>H. elliptica</i>	Stem	4.97	0.08
<i>P. pyrifolia</i> var. <i>culta</i>		4.47	0.04
<i>C. maxima</i>		4.02	0.06
<i>M. pumila</i>		3.94	0.06

and changed to the structural B, which leads to greater $\delta^{11}\text{B}$ values in structural B. The level and the difference of $\delta^{11}\text{B}$ within plants are dependent on the height and the growth period of the plant species. Thus, with an increase in the height and the growth period of a plant, the greater the $\delta^{11}\text{B}$ values.

However, B isotopic compositions in aboveground organs were differentiated between the tissues of the plant parts, such as in stem-leaf and stem-flower in Fig. 2, which lead to lower $\delta^{11}\text{B}$ level in the stem of *W. florida* (A, B, and C), *S. musсотii* (E and F), in which the variations of B isotopic compositions were similar to those reported by Xu et al. (2015). It was inferred that among developmental plant periods, the physiological and biochemical function of plants in most plant species required more B for reproductive growth than for vegetative growth. This would lead to the accumulation in the given tissue (such as lower $\delta^{11}\text{B}$ in stem and greater $\delta^{11}\text{B}$ in flowers, as shown in Fig. 2), which results in significant changes of B mass fractions and isotopic compositions in different organs.

Based on the inference that the trigonal ^{11}B -enriched $\text{B}(\text{OH})_3$ was preferentially transported to the young plant part by the nutrient transporter (Geilert et al., 2016), the maximum $\delta^{11}\text{B}$ value should occur in the fruit or seed compartment. In the observed results, $\delta^{11}\text{B}$ values in the two forms of the three fruits showed a reversed trend compared with in other plant samples, in which $\delta^{11}\text{B}$ values in hydro-soluble form were greater than in the structural form. The calculated α values in these plant organ samples should be lower than 1, indicating that more ^{10}B in the form of $\text{B}(\text{OH})_4^-$ enters the cell wall in structural B form. The variations in α values in the samples, which ranged from 0.9954 to 1.0150, are shown in Fig. 2. In Fig. 2, only the three fruit samples in the dotted area had α values lower than 1, indicating a consistent conflict with the theory. In the other samples, the α were all greater than 1, indicating that more ^{11}B in the form of $\text{B}(\text{OH})_3$ entered structural B, resulting in more ^{10}B in the form of $\text{B}(\text{OH})_4^-$ being left in the sap. A possible explanation can be used to explore the reverse fractionation of B isotope in fruits. In the fruit growth period, the light B isotope was preferentially bound with the cis-polyols at a higher concentration in the sap, in which large amounts of B were used for reproductive growth.

3. Conclusion

An analysis of the hydro-soluble and structural B in plant samples, allowed the measurements of the B isotope compositions in the two forms by MC-ICP-MS, which enables the application of B isotopes in biogeochemistry, plant nutrition, and plant metabolism. The observed B isotopic fractionation in same plant organ indicated that a reversal in the fractionation of B isotopic composition occurred in these samples, meaning that both two B isotopes can be preferentially enriched in cell wall in some organs, which was demonstrated by α values. The compositional variation and α value of the B isotope observed in the present study can be used to investigate the functions and transport mechanisms of B in biological systems. Moreover, knowledge of the cycling, accumulation, and isotopic fractionation of B during the uptake and utilization in intra-plant or interplant situations

will contribute to a better understanding the global biogeochemical cycle of B.

4. Materials and methods

4.1. Information of plant species

The collected samples used to investigate the variation of B isotopic compositions includes *Weigela florida* cv. Red Prince, *Swertia musсотii* Franch., *Cynomorium songaricum* Rupert., and three fruits, *Pyrus pyrifolia* var. *culta* (Makino) Nakai, *Citrus maxima* (Burm) Merr., and *Malus pumila* Mill. *S. musсотii* was collected in the Qinghai-Tibet Plateau area, *W. florida* in Shandong area, and *C. songaricum* in Inner Mongolia area, China. The three fruits were purchased in the local market. For the root holoparasite *C. songaricum*, only stem and flower samples were obtained. The information on sample sites, plant species and soil conditions in the regions are summarized in Table 4.

4.2. Reagents

Hydrochloric acid (Guaranteed Reagent) was redistilled to remove exogenous B and was stored in a sealed polypropylene vessel. The isotopic reference standard used in this study was NIST SRM 951 boric acid (NIST, Gaithersburg, MD, USA).

High purity water with a B blank of $<0.012 \mu\text{g}$ was redistilled by sub-boiling distillation, passed through a column filled with B-selective resin (Amberlite IRA 743), and then used to prepare the standard and working solutions.

4.3. Apparatus

An inductively coupled plasma optical emission spectrometer (ICP-OES, Vista MPX, Varian, USA) with a 40-MHz radio frequency generator and a charge-coupled device detector was used for the determination of B (Sun et al., 2014). The spectral line for the B determination was set at 249.68 nm.

A Neptune plus MC-ICP-MS (Thermo Fisher Scientific, Waltham, MA, USA) was employed for B isotope measurement, coupled with a double-focusing magnetic sector field mass spectrometer equipped with nine Faraday cups and four ion counters, enabling static measurements of m/z 10 and m/z 11. The B ratios were measured using the standard-sample bracketing technique, with NIST SRM 951 H_3BO_3 as the isotopic standard (Wei et al., 2013).

4.4. Separation of B in the two forms from plant species

About 0.2–0.3 g of dried plant sample was weighted and placed into a solid-liquid phase extraction apparatus. Then, 2.5 mL deionized water was added to the apparatus. The apparatus with the sample and extraction solution, was placed in an ultrasonic stirrer with water-bath at 35 °C for 20 min. Then the apparatus was immersed into a 10 mL-centrifuge tube, and centrifuged at 5000 rpm for 5 min to separate the residual sample and supernatant. The process was performed five times. All of the collected eluates were combined in a 30-mL Teflon tube, and evaporated at

Table 4
Information of sampling sites and plant species.

Species	Sampling location	Altitude (m)	Longitude	Latitude	Soil type
<i>W. florida</i>	Linyi, Shandong	71	118° 17' 13.21"E	35° 6' 21.24"N	Shantung soil
<i>C. songaricum</i>	Jilantai, Inner Mongolia	1060	105° 37' 13.83"E	39° 34' 42.61"N	Sandy soil
<i>S. musсотii</i>	Yushu, Qinghai	3585	97° 53' 23.28"E	33° 20' 12.12"N	Alpine steppe soil

40 °C with an air stream. When the remaining solution was reduced to ~1 mL, it was quantitatively transferred to a 10-mL quartz crucible (Sun et al., 2017). The solution in the quartz crucible was evaporated at 40 °C to ~0.1 mL and then transferred to a muffle furnace for dry ashing at 600 °C for 4 h. Finally, after the extraction of hydro-soluble B, the residual plant particles were also transferred to the quartz crucible for the separation and analysis of B according to the method reported by Xu et al. (2015).

4.5. B isotope measurement

The B isotopic composition of the sample is expressed as $\delta^{11}\text{B}$ (‰) relative to that of the NIST SRM 951 H_3BO_3 standard as shown in Eq. (4):

$$\delta^{11}\text{B} (\text{‰}) = [({}^{11}\text{B}/{}^{10}\text{B})_{\text{Sam}}/({}^{11}\text{B}/{}^{10}\text{B})_{\text{Std}} - 1] \times 1,000, \quad (4)$$

where $({}^{11}\text{B}/{}^{10}\text{B})_{\text{Sam}}$ and $({}^{11}\text{B}/{}^{10}\text{B})_{\text{Std}}$ represent the B isotopic ratios of the sample and the NIST SRM 951 H_3BO_3 standard, respectively.

The α value of the B isotopes was calculated in Eq. (5) as follows:

$$\alpha = (1000 + \delta^{11}\text{B}_{\text{Str}})/(1000 + \delta^{11}\text{B}_{\text{Hyd}}), \quad (5)$$

where $\delta^{11}\text{B}_{\text{Str}}$ and $\delta^{11}\text{B}_{\text{Hyd}}$ represent the $\delta^{11}\text{B}$ values of structural and hydro-soluble B, respectively.

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