Variations in the boron isotope composition of *Coffea arabica* beans

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Abstract

Significant B isotope abundance variations were found for a variety of *Coffea arabica* beans from a number of coffee-growing regions around the world. This may be attributed to the influence of local sources of B (including soil, water and fertilizer) each having a characteristic B isotope abundance ratio of its own. The results of this preliminary study indicate that B isotope abundance data can be used to study the biogeochemical cycle of B, an important micronutrient. In addition, the isotopic data can be employed in quality assurance programs of commercial coffee as the quality depends to a large extent on the genetic and geographic origin of the coffee. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Boron is recognized as an essential element for the healthy development of many plant species. Although the exact biochemical role of B in plant growth is not yet well understood, a number of metabolic processes (i.e. carbohydrate metabolism, hormone action, membrane function and nucleic acid synthesis) have been shown to be affected by the amount of plant-available B (Lovatt and Dugger, 1984). Boron deficiencies are common in agricultural soils and trace quantities of this element are often applied during the cultivation of food crops as a foliar spray or fertilizer supplement (Gupta, 1979).

Early investigations of the impact of B fertilization to coffee growth determined nutritive and toxic levels of B by measuring its concentration in coffee leaves and soils (e.g. Chaverri et al., 1957). Coffee was grown in unfertilized and B-deficient lots and in lots where increasing levels of B were added to induce toxicity. Extensive studies conducted by researchers at the Coffee Research Centre farm (CICAFE) in Costa Rica resulted in a large amount of data on the relative concentrations of B in different generations of leaves and the soils where the coffee was grown. The CICAFE researchers identified optimal foliar B concentrations to be above 100 ppm, critical levels between 40 and 60 ppm and deficient levels below 40 ppm (Ramírez, 1992; personal communication, 1997). Thus, an empirical "coffee formula" was adopted by coffee growers whereby a small amount of B is added to the soil. Despite the improved yields of higher quality coffee, the concentration data could not discern the physiological basis of B in coffee growth. This is due to the fact that the concentration data by itself was incapable

of identifying the different sources of B in the region and their relative contributions to the plant. In contrast, the source-specific nature of B isotope abundance data in combination with elemental concentrations appear to be sensitive parameters for tracing the source(s) of this element and its path as it moves through the environment.

Boron has two stable isotopes of mass 10 and 11 (roughly 20 and 80% natural abundance, respectively). A dominant B isotope fractionation mechanism in nature is the pH dependent isotope exchange between boric acid $\{B(OH)_3\}$ and borate ion $\{B(OH)_4^-\}$ such that the heavier isotope of B is favoured in the boric acid and the borate ion is relatively depleted in ^{11}B. The equilibrium constant (K) for this exchange (Eq. 1) is 1.0206 at 0°C decreasing slightly to 1.0177 at 60°C (Kakihana et al., 1977).

$$^{10}B(OH)_3 + {}^{11}B(OH)_4^- \overset{K}{\leftrightarrow} {}^{11}B(OH)_3 + {}^{10}B(OH)_4^- \qquad (1)$$
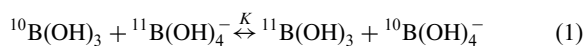
The boric acid dissociation constant (pK_a) between boric acid and borate ion is 8.830 at 25°C and increases with increasing temperature (Eq. 2).

$$B(OH)_3 + H_2O \overset{pK_a}{\leftrightarrow} B(OH)_4^- + H^+ \qquad (2)$$
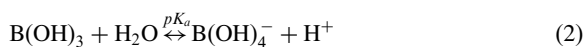
Boron is stable in aqueous solutions as an oxyanion and is not affected by oxidation-reduction reactions.

Boron isotope abundance ratios are reported as δ values calculated with respect to the Standard Reference Material SRM 951 (Eq. 3). SRM 951 is a boric acid standard from Searles Lake, CA and has a certified $^{11}B/^{10}B$ isotope abundance ratio of 4.04362 ± 0.00137 (Catanzaro et al., 1970).

$$\delta^{11}B(\text{‰}) = \left[\frac{\left(\frac{^{11}B}{^{10}B}\right)_{sample}}{\left(\frac{^{11}B}{^{10}B}\right)_{SRM951}} - 1 \right] \times 1000 \qquad (3)$$

Boron exhibits isotope abundance variations greater than 90‰ in nature (Bassett, 1990; Barth, 1993; Aggarwal and Palmer, 1995; Palmer and Swihart, 1996). Significant B reservoirs include ocean water, evaporite minerals and clays. Boron in ocean water has a globally uniform elemental concentration of 4 mg/l and a $\delta^{11}B$ value of +40‰ (Agyei, 1968; Schwarz et al., 1969; Spivack and Edmond, 1987; Bassett, 1990). The ^{11}B enrichment of ocean water is thought to be the result of the adsorption and incorporation of borate anions (enriched in ^{10}B) onto clay and basalt and the low temperature alteration of carbonate minerals and the oceanic crust. Evaporite minerals exhibit a large range in B isotope composition (from −30 to +30‰) depending on the $\delta^{11}B$ value of the B in solution and

the pH of the fluid from which the minerals were precipitated. For example, borate minerals from marine environments are more enriched in ^{11}B than those of non-marine origin (Oi et al., 1989).

The B contents and isotope compositions of ground waters are variable and depend on the source(s) from which B is derived including the leaching of country rocks, mixing with adjacent aquifers, inputs from aerosols and anthropogenic activities. Vengosh et al. (1991) analyzed water from the Great Artesian Basin in Australia and obtained $\delta^{11}B$ values ranging from −15.9 to +2.2‰. The depleted ^{11}B content of these waters suggested that B was leached from surrounding rocks of non-marine origin. In contrast, ground water from the Rift Valley in Israel was comparatively enriched in ^{11}B with $\delta^{11}B$ values ranging from +14 to +44‰ (Vengosh et al., 1994). The variations in $\delta^{11}B$ values in the ground waters from the Rift Valley were the result of mixing of subsurface brines with $\delta^{11}B$ values greater than +40‰ and high temperature water–rock interactions involving carbonates and clay minerals with $\delta^{11}B$ values less than +14‰.

Boron is present only as a trace element in the atmosphere with a maximum concentration of 10 ng B/m³. Boron in the atmosphere originates from the outgassing of volcanoes, agricultural burning and forest fires, industrial emissions including fuel burning, and to a lesser degree from the volatilization of oceanic B (Fogg and Duce, 1985; Nishimura and Tanaka, 1972). Spivack (1986) reported B concentrations and isotope compositions of rainwater and dry deposition collected from 3 sampling stations on the islands of Guam, Enewetak and Fanning located between the equator and 15° North in the Pacific Ocean. The samples had B concentrations ranging from 3 to 16 ppb and $\delta^{11}B$ values between +0.8 to +35.0‰.

In contrast to geochemical systems, there have been few measurements of the B isotope compositions of plants. Vanderpool and Johnson (1992) report a range in $\delta^{11}B$ values from −7.5 to +29.3‰ for a variety of commercial produce grown in different regions of North and Central America. These authors also investigated the incorporation of ^{10}B-enriched B (95.91 atom % ^{10}B) by broccoli and cabbage plants. They compared the $\delta^{11}B$ values of broccoli and cabbage grown in (1) a soil-less mixture with a commercial fertilizer (of unspecified B isotope composition), (2) plants grown in a soil-less mixture with a ^{10}B-enriched solution applied as a foliar spray and (3) plants grown hydroponically with a ^{10}B-enriched nutrient solution. The broccoli and cabbage grown in a soil-less mixture with a commercial fertilizer had $\delta^{11}B$ values of −6.3 and −2.3‰, respectively. Broccoli and cabbage grown with foliar and hydroponic solutions enriched in ^{10}B had much lower $\delta^{11}B$ values compared to commercial produce. Both the broccoli and cabbage grown in a

soil-less mixture with the ^{10}B-enriched foliar spray had δ^{11}B values of −543 and −568‰, respectively. The plants grown with the ^{10}B-enriched hydroponic nutrient solution had δ^{11}B values of −969 and −976‰. However, the plants did not acquire the same B isotope composition as the applied ^{10}B-enriched solutions. The observation that the plant tissues did not have identical δ^{11}B values as the nutrient solutions may suggest that B isotopes were fractionated during incorporation into the plants.

The present study of the B isotope composition of *Coffea arabica* beans was motivated by the need to evaluate agricultural practices currently in use in different coffee agroecosystems. The export of coffee is of significant economic importance for many countries and considerable emphasis is placed on improving the quality and quantity of crop yields. The quality of coffee depends to a large extent on the genetic and geographic origin that gives the product specific physical, chemical and organoleptic characters. Predicting the quality of commercial coffee is no easy matter and well-established and scientifically sound methods are still lacking (Barrel and Jacquet, 1994). Thus, quantitative methods to assess the quality of coffee beans are required to verify the origin of the product. Isotopic signatures may be important tools in this exercise as they are good markers of environment (soil and ground water) and agricultural practice (irrigation and fertilization). This paper reports the results of a feasibility study in the application of B isotopes to assess the role of this micronutrient in the cultivation of *Coffea arabica*. This preliminary investigation indicates that B isotopic signatures could be effective tools in the improvement of agricultural practices including irrigation methods and the preparation and application of fertilizers as well as the assessment of commercial coffee quality.

2. Experimental

A selection of 14 unroasted *Coffea arabica* bean samples representing a variety of coffee-growing regions from around the world were obtained from a local coffee-roasting company. Coffee samples were dissolved by microwave digestion (Vanderpool and Johnson, 1992; Evans and Krähenbühl, 1994) and the B was isolated from the digested sample using a 10 cm length of 1 mm ID Teflon® column filled with 80 mesh Amberlite IRA-743 B specific resin (Hemming and Hanson, 1994). The B-containing solution eluted from the ion exchange column was evaporated to dryness at 60°C. Samples of SRM-951 (boric acid Standard Reference Material supplied by the National Institute of Standards and Technology, Gaithersburg, Maryland, USA) were also subjected to the same microwave

digestion procedure as the coffee samples to ensure that the method did not cause B isotope fractionations. The mean ^{11}B:^{10}B ratio measured for SRM 951 in this work was 4.0337 ± 0.0041 (1s, $n = 10$).

Boron isotope abundance measurements were performed using negative ion thermal ionization mass spectrometry (Zeininger and Heumann, 1983) on an extensively modified AVCO thermal ionization mass spectrometer (Wieser, 1998). The dried residue of the B containing solution eluted from the ion exchange column was redissolved in 5 μl of a 2 μg/μl Ba solution (Ba(OH)$_2$ dissolved in 1 M HCl) and deposited on a single outgassed Re filament (4 pass zone-refined, 99.999 % Re). The sample-coated Re was placed into the mass spectrometer ion source and the pressure reduced to less than 10^{-7} Torr. The filament was then heated gradually to 950°C at which point ion currents at mass 42 (^{10}BO$_2^-$) and 43 (^{11}BO$_2^-$) were measured. Typically, 10^{-11} A ion currents were produced from 100 ng of B. One hundred ^{11}B:^{10}B ratios from each sample were measured and the average isotope abundance ratio was calculated. Replicate measurements of the sample (including microwave digestion and ion exchange separation) resulted in B isotope ratios with precisions of ±0.3% (2s). Isobaric interference at mass 43 due to ^{17}O-isotope interference (i.e. ^{10}B^{16}O^{17}O$^-$ and ^{11}B^{16}O^{16}O$^-$) may increase the measured 43:42 ion current ratio by 0.00076 (Heumann and Zeininger, 1985). However, typical analytical uncertainties in this work were an order of magnitude larger than this offset and a correction to the measured 43:42 ion current ratio was not applied. A potential isobaric interference at mass 42 from ^{12}C^{14}N^{16}O$^-$ was monitored by measur-

Table 1

Boron concentration and isotope composition of coffee bean samples from different coffee growing regions

Region	B concentration (μg/g)	δ^{11}B (‰) ±3‰ (2s)
Peru ("organic")	14.5	−12
New Guinea	11.5	−5
Guatemala	12.1	−3
Columbia	12.2	−2
Brazil	14.4	−2
Mexico	13.8	−1
Hawaii	12.1	−1
Costa Rica	10.5	0
Nicaragua	11.3	0
Celebes	15.3	+1
Java	13.6	+15
Ethiopia	9.5	+15
Mexico ("organic")	14.2	+18
Tanzania	10.7	+19

ing the ion current at mass 26 ($^{12}C^{14}N^-$). The presence of CN^- indicated that significant quantities of organic compounds were still present in the sample and not removed sufficiently during the digestion/ion exchange procedure. Samples that exhibited this organic interference were reanalyzed by digestion and ion exchange separation

Boron concentrations were measured by ICAP-AES at the Instituto de Pesquisas Energeticas e Nucleares, São Paulo, Brazil. Less than 200 mg of finely powdered (< 100 mesh) coffee was digested with 10 ml of concentrated HNO_3 (purified by sub-boiling distillation) in a Teflon® PFA digestion vessel. The precision and accuracy of the B concentration analyses were better than 10%.

3. Results and discussion

The measured $\delta^{11}B$ values for the various coffee beans varied from −12 to +19‰ (Table 1). In contrast, B concentrations were much more uniform and averaged 12.4 ± 1.9 μg/g (Table 1). No correlation between the B concentrations and isotope compositions ($r^2 = -0.1$, $n = 14$) was observed.

The measured variation in $\delta^{11}B$ values among the different coffee beans is significant and is likely due to different local growing conditions. The 3 major inputs of B to the coffee plant are the soil, the water supply and fertilizer. The total B content of many soils ranges from 7 to 80 μg/g with B being concentrated in minerals such as tourmaline, and in clays, shales and organic matter (Evans and Sparks, 1983). Boron bound in tourmaline is not leached easily as it is held tightly in the mineral structure (Gupta, 1968). In contrast, B in organic matter can be more easily released by microbial activity. However, this release is dependent on the soil's moisture content and pH. Under drought conditions, the microbial activity in the soil decreases and the B remains complexed and unavailable to the plant. As the soil moisture content increases the B uptake by plants increases and is positively correlated to the pH of the soil (above a pH of 6.3). Presumably, as the pH increases the adsorption of B by clays reduces the amount available that can be leached readily.

A significant portion of B in plants is derived from fertilizers that are applied to soils with concentrations ranging from 0.3 to 3 kg B/ha (Gupta, 1979). In a recent study, Komor (1997) measured the B content and isotope composition of 4 different fertilizers including ammonium nitrates, urea and phosphate-based products. The author found B concentrations varied from less than 0.46 to 22.40 mg/l. Boron isotope compositions ranged from −2.0 to +14.8‰. The range in the $\delta^{11}B$ values of fertilizers is of significance

because the $\delta^{11}B$ value of the applied fertilizer will have an effect on the B isotope composition of the plant tissue (Vanderpool and Johnson, 1992). Boron minerals such as borax, colemanite and kernite are processed to produce B fertilizers and the $\delta^{11}B$ value of the mineral used will reflect the B isotope composition of the fertilizer. Therefore, the range of $\delta^{11}B$ values in fertilizer found by Komor (1997) may reflect the isotopic composition of the B obtained from different mining locations. At present, almost half of the world's refined borax comes from sodium borates at Boron in California's Mojave Desert with additional mining operations located in the Argentinean Andes, Chile and Peru (Sevey, Borax Inc., personal communication, 1999). Borax from Boron, California, USA has a $\delta^{11}B$ value of +0.3‰, ulexite from Argentina has $\delta^{11}B$ values as negative as −31‰ and B minerals from Turkey have $\delta^{11}B$ values ranging from −14.9‰ in colemanite to −1.6‰ in borax (Palmer and Helvaci, 1995).

A possible influence of the fertilizers on coffee beans can be seen in two samples from Central America that were labeled as "organically grown" (one from Mexico the other from Peru). These coffee beans had distinct $\delta^{11}B$ values compared to the $\delta^{11}B$ values of coffee from neighbouring regions that utilized B-containing fertilizers. In Mexico, the organically grown coffee had a $\delta^{11}B$ value +18‰ compared to a $\delta^{11}B$ value of −1‰ for coffee grown conventionally from the same region. Both types of coffee had near identical B elemental concentrations of approximately 14 ppm (Table 1). Although the specific agricultural methods used to grow these coffee plants are not available, previous studies on the comparative economics of organic and conventional coffee cultivation state that borax-containing fertilizers are not added to organically grown plants (Pülschen and Lutzeyer, 1993).

4. Summary

Significant $\delta^{11}B$ variations were found among *Coffea arabica* bean samples from different coffee growing regions around the world. This suggests that the isotopic composition of local B sources (i.e. B in the soil, fertilizer and water supply) control the $\delta^{11}B$ values of the coffee beans. The sources of B to the plant are many and each may have a characteristic B isotopic composition. Boron is not readily translocated once bound in the plant tissue. Therefore, coffee beans will have a B isotope composition that represents the cumulative input of the various sources. This study indicates that B isotope abundance and elemental concentration data can be employed to study the biogeochemical cycle of B in the growth of coffee plants. Yet another application of B isotope data lies in the commercial aspects of coffee production, namely

quality assurance. The genetic and geographic origins are largely responsible for the quality of the coffee. Such being the case, any parameter (for example $\delta^{11}B$ data) that can indicate the geographic origin is worth investigating.

Acknowledgements

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