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Isotope signature study of the tea samples produced at four different regions in India

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Rupali A. Lagad,[a] Devanathan Alamelu,[a] Amzad H. Laskar,[b] Vinai K. Rai,[b]
Sunil K. Singh[b] and Suresh K. Aggarwal*[a]

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India ranks second in the world for tea production and is well known for Darjeeling tea, which has great demand in the world market due to its unique flavor. In the present study, the combination of isotopic composition of Sr (as $^{87}Sr/^{86}Sr$) and C (as $\delta^{13}C$) was studied as geographic tracing signatures for Indian tea samples grown in different regions. Authentic tea leaves as well as soil samples were collected from different tea producing regions; namely, Assam, Darjeeling, Munnar and Kangra, which are geographically distinct from one another. Isotopic analyses were performed by Multi-Collector Inductively Coupled Plasma Mass Spectrometry and Elemental Analyzer-Isotope Ratio Mass Spectrometry for Sr and C, respectively. On the basis of Sr isotopic data, Darjeeling tea samples were found to be more radiogenic than the other tea samples, with changes in the $^{87}Sr/^{86}Sr$ ratio being an excellent geographic indicator. Variations in $\delta^{13}C$ proved to be an excellent geographic indicator for Munnar and Kangra teas. The $^{87}Sr/^{86}Sr$ values were statistically identical in both the soil and the tea. Principal Component Analysis (PCA), using the combination of $^{87}Sr/^{86}Sr$, $\delta^{13}C$ and strontium concentration data, was used to effectively differentiate among different tea producing regions.

1 Introduction

The market for imported, premium priced foods has increased dramatically over recent years as consumers become aware of products originating from around the world. There are many food products that are of superior quality (taste, texture, fragrance, *etc.*) because of the locale in which they are cultivated. Environmental conditions, such as local climate and soil characteristics, combine to yield crops that exhibit specific traits. Clearly, better quality fetches higher market prices. Therefore, unscrupulous traders may attempt to increase their profits by deliberately mislabeling foods, or by increasing the volume of a good quality batch through adulteration with the sub-standard produce. The quality of foods is of primary importance for both the consumers as well as the producers. As per country food legislation, quality standards have been established through the requirement of quality labels that specify the chemical composition of each product, and its authentication is essential to avoid unfair competition that can create a destabilized market.

Darjeeling, the North-East part of India, is well known for tea of unmatched flavor and quality, which sets it apart from other teas in the world. As a result, it has won the patronage and recognition of discerning consumers worldwide for more than a century. Darjeeling tea by definition cannot be grown or manufactured anywhere else in the world except Darjeeling. About 10 million kilograms of tea production every year is taken from 87 tea gardens grown at over 5000 meters above sea level spread over 17 500 hectares of land in 4 flushes per year. In order to avoid adulteration and forgery of Darjeeling tea samples, tea produced in India is administered by the Tea Board of India under the Tea Act of 1953. On a worldwide basis, the Tea Board of India has given a Geographical Indication to Darjeeling tea for its protection and preservation of its treasured icon of India's cultural heritage. Along with the Darjeeling tea, the Tea Board of India has assigned a geographical logo to other known regions tea such as Assam tea, Kangra tea and Nilgiri tea. The labeling of the geographical logo on the final product is considered to be the authenticity of the product in the market and is legislatively compulsory for all tea manufacturers in India under the Tea Act of 1953.[1]

Several scientists working in food research are finding answers to these food adulteration problems. One of the approaches being tried is to determine variations in the isotopic ratios of elements such as H, C, N, S, B, Sr, and Pb in various food matrices because these isotopic ratios can vary according to the geographical origin of the matrix. Therefore, different isotope ratios can be used in fingerprinting techniques for food authentication.

Local agricultural practices and the environment affect $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios, respectively. C_3 plants use the Calvin

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[a] *Fuel Chemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India. E-mail: skaggr2002@rediffmail.com; skaggr2002@gmail.com; Fax: +91 22-25505151/5345; Tel: +91 22-25593740*

[b] *Physical Research Laboratory, Navrangapura, Ahmedabad 380 009, India*

photosynthetic pathway to assimilate CO_2.[2] During this process, the plants discriminate against ^{13}C and, therefore, possess relatively lower $^{13}C/^{12}C$ ratios than C_4 plants that utilize the more energy efficient Hatch–Slack pathway.[2] Since C_3 plants predominate at higher latitudes and C_4 plants are more common in warmer climates at lower latitudes (such as the tropics), there is a gradient of a decreasing $^{13}C/^{12}C$ ratio in plant materials from the equator to the poles, which can also be used as a proxy for geographical origin determination. In the present study, tea plant leaves used for analyses belong to C_3 plant groups.[2]

Similarly, the $^{87}Sr/^{86}Sr$ isotope ratio is one of the most popular among the potential isotopic systems which provide fingerprint data depending on the geographical origin. Strontium has four naturally occurring isotopes. Three of them (^{84}Sr, ^{86}Sr, and ^{88}Sr) are non-radiogenic. The fourth, ^{87}Sr, comes, in part, from the natural β-decay of ^{87}Rb and its concentration in the minerals depends on the age of the rock and on the Rb/Sr ratio. This makes the $^{87}Sr/^{86}Sr$ isotope ratio an important parameter in geochronology. The $^{87}Sr/^{86}Sr$ isotope ratio was demonstrated not to change during biological processes and reflects the environment of growth: bedrock, soil, and soil–water in plants.[3] Horn *et al.* (1993, 1998) reported that the $^{87}Sr/^{86}Sr$ ratio fell into the respective ranges for country rocks and respective soils.[4,5]

Several research studies on stable isotope ratios, especially those of the light elements $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{18}O/^{16}O$, were reported for determining the authenticity of food products such as butter,[6] lamb meat,[7] honey,[8] Scotch whisky,[9] and single seed oils.[10] The heavy element isotope ratios $^{87}Sr/^{86}Sr$[4,11] and lead isotopic composition[12,13] were applied to the differentiation of wine according to the geographical origin. $^{87}Sr/^{86}Sr$ ratios were used in provenance studies on rice[14] and cheese.[15]

In order to develop a method to authenticate the Indian teas, it was of interest to check the variations in the carbon and strontium isotopic ratios of authentic raw tea leaves obtained from different tea gardens of Indian regions (Assam, Darjeeling, Munnar and Kangra), and compare these data with C and Sr isotopic composition variation found for processed tea samples available in the local market and develop a strategy for identifying the origin of the tea produce.

2 Experimental

2.1 Materials and methods

Milli-Q water (Milli-Q™, Purification device, Millipore Corporation, Bengaluru, India) was used throughout the analysis. Suprapure grade (Merck) reagents *e.g.* 65% HNO_3, 50% H_2O_2, 35% HCl and 40% HF were used for digestion. All reagents used for Sr chemistry were purified by sub-boiling distillation in a Teflon/Quartz distillation still. Separation of Sr from the digested tea samples and the soil samples was carried out using Sr specific resin (M/s. Eichrom) and Dowex-AG50xW cation exchange resin, respectively. Multi-elemental ultrapure ICP standard solution (1000 mg L^{-1}, Merck, Darmstadt, Germany) was used for preparing Sr solutions for calibration. Sr isotopes in NIST SRM 987 (50–200 ppb) were measured at regular intervals during the analytical run. IAEA-Sucrose-CH-6 standard of certified $\delta^{13}C$ value w.r.t. VPDB was used for quality control.

2.2 Tea samples and sample preparation

Thirty-eight samples of Indian tea were collected from different tea gardens of Assam (16), Darjeeling (11), Munnar (Kerala) (6), and Kangra (5). The different tea producing regions of India are shown in Fig. 1. Authentic tea leaves collected from the tea gardens were first washed with Milli-Q water to remove any dust particles deposited on the leaf surface and were air dried. Dried tea leaves (10–15 g) were ground in an agate mortar and pestle and were sieved through a 120 mesh to obtain a fine homogenized powder before analyses.

For carbon isotope ratio measurements, 100 μg of this homogeneous powder was weighed into a tin capsule (5 × 9 mm) which was folded and compressed to contain the sample and minimize the presence of any air. Each sample was packed in triplicate. Standard reference material IAEA-Sucrose-CH-6 having a certified $^{13}C/^{12}C$ value was also packed and used for method validation.

For determining the total Sr content and Sr isotope ratios, 250 mg of the homogenized tea sample was digested using a closed vessel microwave digestion system (Milestone-3000) fitted with Teflon vessels. A mixture of 3 mL of 65% Suprapure HNO_3 and 1 mL of 50% H_2O_2 was used to obtain a clear solution free from the organic matrix.[16] The clear solution obtained was finally diluted to 25 mL with 1 M HNO_3. For Sr isotopic composition, digested samples were evaporated by heating at 80 °C until the volume was reduced to approximately 1 mL. Evaporation was performed in open Savillex vials on a temperature controlled hot plate, in a laminar flow hood, to avoid any contamination of the samples.

2.3 Soil samples and sample preparation

Soil samples were also collected from the same tea plantation and from the same plant for which leaves were taken for analyses. About 150 g of the soil sample was collected from different tea plantations of Assam, Darjeeling and Kangra regions. Soil samples were collected at a depth of 10 to 20 cm from the surface and from the root zone area. The soil samples were preserved in pre-cleaned polyethylene bags and transported to the laboratory. The soil samples were disaggregated and dried in an oven at 60 °C for 10 h followed by grinding in an agate mortar and pestle. The powdered samples were sieved using a 120 mesh screen, and homogenized and stored in polyethylene bags until analyses. Sixteen representative soil samples were selected for Sr isotopic determination on the basis of their collection locations.

Several studies are reported in the literature to decompose specific components of the soil using hot plate or microwave digestion.[11,16–19] In the present study, to determine Sr isotope ratios in soil samples, 100 mg of the homogenized soil sample was digested in Savillex vials (7 mL capacity) using a temperature controlled hot plate in a clean laminar flow hood to avoid contamination. For digestion, a mixture of high purity concentrated 4 mL of HNO_3 and 2 mL of HF was used twice,

Fig. 1 Tea producing regions of India.

followed by treatment with 4 mL of aqua regia. Digestion was carried out at ~100 °C until a clear solution was obtained, which was then evaporated to dryness and redissolved in 1.5 mL of 2 M HCl.[19]

2.4 Strontium and rubidium separation

Separation of Sr from Rb and other matrix components of digested tea leaves and soil samples was finally carried out by using a strontium specific extraction chromatographic Sr Spec™ resin (M/s. Eichrom) and Dowex-AG50xW cation exchange resin, respectively. Although the use of Sr Spec resin for the separation of Sr from other matrix components is one of the known methodologies available in the literature,[20,21] in the present study, the separation of Sr from Rb and other matrix components in geological samples was carried out using Dowex-AG50xW cation exchange resin, in view of the higher contents of Sr and other salts in soil as compared to those in the tea leaves.[19,22] For strontium separation, the following procedure was employed.

Recovery of Sr from tea leaves digested solution was carried out using Sr Spec resin by preparing a synthetic mixture containing K, Al, Ca, Mn, Mg and Sr with concentrations in the range of those present in the tea samples. The elution patterns of Sr and the other elements were studied. The Sr recovery in the synthetic mixture was found to be >95% and the interfering elements were found to elute in the first washing. Strontium separation was also checked using Sr Spec resin with reference material SRM-987 which gave a Sr recovery of >99% (ref. 20 and 21) and the determination of the ^{87}Sr/^{86}Sr isotopic ratio was within the certified range (0.71022–0.71030). The entire separation procedure for actual tea samples was designed based on this study.

For actual tea samples, pre-cleaned Sr specific resin was loaded in polypropylene micro-columns up to 0.2 mL bed height of the resin. To avoid memory effects, fresh Sr Spec resin was used each time and the micro-columns were pre-cleaned thoroughly by soaking them in acid and Milli-Q water. Resin was washed with 3 × 1 mL of Milli-Q water and conditioned with 3 × 1 mL of 8 M HNO_3. The dried sample was re-dissolved with 500 μL of 3 M HNO_3 and the solution was loaded onto preconditioned Sr Spec resin in a polypropylene column (200 μL). Major matrices were removed by washing with 3 mL of 3 M HNO_3. The Sr fraction was eluted using 4 mL of Milli-Q water and acidified with 2% HNO_3 (v/v) for the MC-ICP-MS measurement. The recovery of Sr obtained was more than 95% by using this procedure.[20,21]

Similarly, in the case of soil solution, around 1 mL of the digested soil solution was used for Rb–Sr separation. Glass columns were loaded with pre-cleaned Dowex-AG50xW cation exchange resin up to the 10 cm height. Conditioning of resin was carried out by using 2 × 2 mL of 2 M HCl, followed by loading of 1 mL of the digested sample in 2 M HCl. Other separation steps such as loading, washing, collection of Rb, and further washing were carried out using 2 × 1 mL of 2 M HCl, 12 mL of 2 M HCl, 5 mL of 2 M HCl and 14 mL of 2 M HCl, respectively. Finally, the Sr fraction was eluted using 10 mL of 2 M HCl. The Sr fraction was evaporated to dryness and redissolved in Milli-Q water and acidified with 2% HNO_3 (v/v) for the MC-ICP-MS measurements. In this case, the recovery of Sr obtained was more than 90%.[19,22] The SRM-987 was also treated in a similar way as was done for soil digests using Dowex-AG50xW. The recovery of Sr was found to be >90%.

2.5 Instrumentation and measurement

For carbon isotopic composition ($\delta^{13}C$) measurements, the samples and standard reference material were analyzed using an elemental analyzer coupled with an isotope ratio mass spectrometer (EA-IRMS) (Delta V plus interfaced with Flash 2000 Elemental Analyser) (Thermo Fisher). Each sample was run in triplicate. A standard (IAEA-Sucrose-CH-6)–sample–standard (IAEA-Sucrose-CH-6) bracketing method was used to check for any drift in the instrument calibration and other operating parameters.

The total Sr content was determined by using inductively coupled plasma-atomic emission spectrometry (ARCOS, M/s. Spectro, Germany) by taking 5 mL of the digested tea samples. Each sample and a matrix matched certified reference material GSB-7 tea leaves were digested in triplicate and each replicate digested solution was analyzed three times by ICP-AES ($n = 9$). The calibration curve was plotted using 0.1 to 10 mg L^{-1} Sr standard solutions (1000 mg L^{-1}, Merck).

Table 1 Typical instrumental operating parameters for the Neptune MC-ICP-MS during Sr measurements

Neptune MC-ICP-MS parameters			
RF power	1100 W		
Plasma gas flow	14 L min^{-1} Ar		
Auxiliary gas flow	0.8 L min^{-1} Ar		
Nebulizer	PFA 100 nebuliser		
Sampling cone and material	Ni, X-Skimmer cone		
Sample uptake rate	100 μL min^{-1}		
Sampling mode	6 blocks of 12 measurements, run in rotation mode		
Mass analyser pressure	4 × 10^{-9} Torr		
Mass assignment to	Kr	83	L3
Faraday cup detectors	Sr	84	L2
	Rb	85	L1
	Sr	86	Ax
	Sr	87	H1
	Sr	88	H2

Strontium isotope ratio measurements were carried out using a Neptune MC-ICP-MS (Thermo-Fisher, Bremen, Germany). Gain calibration of amplifiers was performed each day before the start of the measurement and was found to be very stable. The machine was tuned to get maximum sensitivity and optimal peak shape before measurement of samples. The samples were diluted for the Sr content so as to obtain the ^{88}Sr signal of 200 mV to 5 V. Each measurement lasting 10 min was followed by a wash sequence with 2% HNO_3 for 2 min, which is enough to bring the Sr signal to the background level. The typical instrument conditions and measurement parameters used are listed in Table 1.

3 Results and discussion

3.1 Method validation

Validation of carbon isotopic ratio measurements for samples was carried out by using a standard–sample–standard bracketing method. The external reproducibility of the mass spectrometer for $\delta^{13}C$ measurement was $\sim 0.1‰$ and this was calculated by repetitive measurement of the IAEA-Sucrose-CH-6 after every 5 samples. The observed average value of $\delta^{13}C$ for IAEA-Sucrose-CH-6 standard was -10.13 ± 0.12 ($n = 30$). IAEA-CH-3 cellulose having $\delta^{13}C$ values closer to the tea leaves is an ideal standard for these measurements but since this standard was not available during these measurements, the results were also validated with respect to NBS Oxalic acid II standard. For cross checking the results, a few of the samples were also analyzed in a Sercon GEO 20-20 dual inlet stable isotope ratio mass spectrometer with respect to a calibrated CO_2 gas (obtained from M/s. Vadilal Gas Company). The results obtained by the two mass spectrometers were within the acceptable uncertainties. A few of the samples were later analyzed using IAEA-CH-3 cellulose as a standard, which was available at a later date, and the results agreed to within 0.1‰.

Similarly, the validation of Sr determination in tea samples was confirmed by analyzing matrix matched certified reference material CRM-GSB-7-tea leaves. Good agreement was observed between the experimentally obtained value (8.5 ± 0.3 μg g^{-1}, $n = 9$) and the certified value (9.1 ± 1.2 μg g^{-1}) of the Sr content, which showed a relative standard deviation of <3% and a *Z*-score value of −0.52.

In the case of Sr isotopic ratio measurement, in order to correct for fractionation during chemistry and measurements, all Sr isotopic measurements were normalized internally with a $^{88}Sr/^{86}Sr$ value of 8.375209. In the present study, the measured range of the $^{87}Sr/^{86}Sr$ ratio of SRM-987 was from 0.71023 to 0.71031 with a mean value of 0.71028 ± 0.00002 ($n = 10$) which was well within the certified range of the $^{87}Sr/^{86}Sr$ ratio of SRM-987, and this agreed well with the range certified for the Neptune tune solution (0.71022 to 0.71030) containing SRM-987 employed in this study.

3.2 Carbon isotopic composition of Indian tea samples

Fig. 2a summarizes the variation of $\delta^{13}C$ in Indian tea samples. The $\delta^{13}C$ values of authentic tea leaf samples varied by

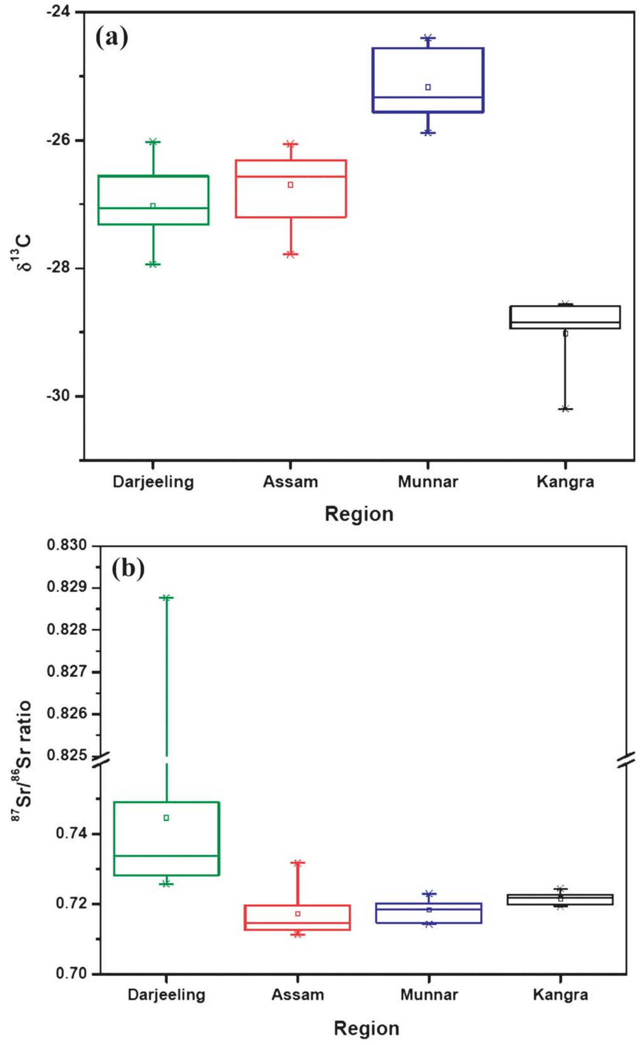

Fig. 2 (a) $\delta^{13}C$ and (b) $^{87}Sr/^{86}Sr$ of tea samples according to the geographical origin in India.

approximately 5.8‰. The mean $\delta^{13}C$ value of the Indian authentic tea leaves was −26.85‰ and it ranged from −24.39 to −30.19‰. The mean $\delta^{13}C$ values of the tea samples from different regions are shown in Table 2. The determination of $\delta^{13}C$ of three replicates of each tea sample showed a maximum standard deviation of 0.12‰. From Fig. 2a, it can be seen that the variations in $\delta^{13}C$ ratios in samples from Assam and Darjeeling are overlapping, whereas Munnar and Kangra regions show distinctly different $\delta^{13}C$ ratios compared to those from Assam and Darjeeling. Therefore, from $\delta^{13}C$ analysis, it is possible to differentiate the tea from the Southern (Munnar), Northern (Kangra) and North-East (Assam and Darjeeling) regions of India. The observed variation in $\delta^{13}C$ values of these three zones may be due to their distinct geographical locations, the effect of sunlight, climate, temperature, levels of precipitations and plant genetic factors on $\delta^{13}C$.[22] Munnar tea samples are from the eastern part of Kerala and is the part of the craggy hills of Western Ghats at an altitude of 1524 meters above sea level and has a latitude of 10°5′0N and a longitude of 77°4′E, whereas the Kangra region is part of the Western Himalayas located at an altitude of 427 to 6401 meters above sea level and has a latitude from 31°2′0N to 32°5′N and a longitude of 77°–77°45′E. These geographical locations of Munnar and Kangra are distinctly different from those of Assam and Darjeeling which are part of the eastern Himalayas. Although, in the present study, $\delta^{13}C$ values of tea samples do not allow differentiation among all the four tea producing regions, it is important to remember that carbon isotopes are one of the most important indicators of ecological changes.[24,25] Ehleringer *et al.* (2000) have demonstrated the impact of environmental factors on $\delta^{13}C$ by carrying out a study on cocaine plants from local geographic farming regions of South America.[26] Similarly, West *et al.* (2009) have shown the effect of environmental growth conditions on $\delta^{13}C$ and $\delta^{15}N$ of Marijuana plants from the United States in their study.[28]

3.3 Concentration and Sr isotopic composition of Indian tea samples

The total Sr concentration of analyzed tea samples ranged from 9.3 to 47.6 μg g^{-1}. The relative standard deviation for the Sr content in each tea sample was less than 3% for ($n = 9$). The concentration range and the mean Sr concentration values with standard deviation of each tea producing region are summarized in Table 2. Concentrations of Sr in all the four tea producing regions were in the order of Assam (18.3 ± 5.1 μg g^{-1}) < Darjeeling (24.5 ± 3.8 μg g^{-1}) < Kangra (31.5 ± 3.8 μg g^{-1}) < Munnar (38.7 ± 4.5 μg g^{-1}).

The variations of $^{87}Sr/^{86}Sr$ ratios for the tea samples from different tea producing regions of India are shown in Fig. 2b in the form of a box plot. The range and mean values of $^{87}Sr/^{86}Sr$ of tea samples for different tea producing regions are summarized in Table 2.

Tea samples collected from the Darjeeling region showed a higher $^{87}Sr/^{86}Sr$ isotopic ratio as compared to tea samples of other regions. This indicates the higher geological age and mineralogy of soil and rock and internally available Sr in the tea plant found in that region compared to those in Assam, Munnar and Kangra regions.[27] The $^{87}Sr/^{86}Sr$ values measured for the Darjeeling region are in the range of 0.726 to 0.829 (with a mean $^{87}Sr/^{86}Sr$ ratio = 0.745 ± 0.029), which is in agreement with the literature data for the corresponding soils and water reservoirs.[29–31] Tea samples from Darjeeling originate from tea plantations with soils developed from granitic and gneissic rocks which have higher values of $^{87}Sr/^{86}Sr$. The observed wide range of $^{87}Sr/^{86}Sr$ ratios in Darjeeling tea samples comprises three sub-regions such as: (i) tea gardens between New Jalpaiguri and Darjeeling (26°44′33.84″N–88°24′14.23″E to 26°48′3.84″N–88°18′18.00″E), (ii) Darjeeling and Kalimpong (27° 3′26.47″N–88°13′59.91″E to 27°3′38.90″N–88°22′37.76″E) regions which showed a higher value of the $^{87}Sr/^{86}Sr$ ratio, and (iii) tea gardens located in the Darjeeling region itself showed comparatively lower values of the $^{87}Sr/^{86}Sr$ ratio. The tea plantations from Darjeeling to Kalimpong regions have $^{87}Sr/^{86}Sr$ values ranging from 0.730 to 0.829 and these are cultivated near the bank of the Teesta River which is among the most radiogenic river reported in the literature having higher $^{87}Sr/^{86}Sr$

Table 2 Data on Sr concentration, ^{87}Sr/^{86}Sr isotopic ratio and δ^{13}C of tea samples originating from different tea producing regions of India[a]

		Authentic tea samples			Soil	
Sampling site	No. of samples	Sr Mean ± SD (μg g^{-1})	^{87}Sr/^{86}Sr ratio Mean ± SD	δ^{13}C Mean ± SD	^{87}Sr/^{86}Sr ratio range Mean ± SD	Soil–plant ^{87}Sr/^{86}Sr correlation coefficient (R^2)
Assam	16(7)	9.3–25.1 18.3 ± 5.1	0.711–0.732 0.717 ± 0.006	−26.05 to −27.78 −26.69 ± 0.56	0.714–0.733 0.720 ± 0.008 [0.718 ± 0.007]	0.98
Darjeeling	11(6)	17.5–28.6 24.5 ± 3.8	0.726–0.829 0.745 ± 0.029	−26.02 to −27.93 −27.02 ± 0.58	0.726–0.797 0.747 ± 0.024 [0.747 ± 0.037]	0.98
Munnar	6(—)	34.5–47.6 38.7 ± 4.5	0.714–0.723 0.718 ± 0.003	−24.39 to −25.88 −25.17 ± 0.57	NA	—
Kangra	5(3)	27.0–37.5 31.5 ± 3.8	0.719–0.724 0.722 ± 0.002	−28.55 to −30.19 −29.02 ± 0.67	0.719–0.728 0.723 ± 0.004 [0.721 ± 0.002]	0.92

[a] Values in ( ) parentheses are the number of soil samples analysed, values in [ ] corresponding tea plant Mean ^{87}Sr/^{86}Sr ± SD, NA – soils samples were not available for analysis.

isotope ratio values of 0.809 to 0.824.[30] This wide range of variation in ^{87}Sr/^{86}Sr implies a difference in the geological age and mineralogy of granitic and gneiss rocks and proportion of carbonate in soil from those regions.

Assam tea samples also showed a wide range of ^{87}Sr/^{86}Sr ratios *i.e.* from 0.711 to 0.732. Tea samples collected from Assam regions, namely Golaghat, Jorhat, Dibrugarh and Sibsagar, showed variations in ^{87}Sr/^{86}Sr ratios. The Golaghat region showed a comparatively higher range of ^{87}Sr/^{86}Sr ratios (*i.e.* 0.725–0.732) than other regions which showed ^{87}Sr/^{86}Sr ratios in the range of 0.711–0.720 (Jorhat), 0.714–0.716 (Dibrugarh) and 0.713–0.719 (Sibsagar). Assam is located at the bank of Brahmaputra and consists of its tributaries, which have shown ^{87}Sr/^{86}Sr isotopic ratios in the range of 0.712 to 0.759. Singh *et al.* (2006) reported data on ^{87}Sr/^{86}Sr ratios to be 0.715 and 0.718 in water and sediment samples, respectively, from the Dibrugarh region, which are closer to ^{87}Sr/^{86}Sr values reported for tea samples from the Dibrugarh region in the present study.[29] Available literature data on ^{87}Sr/^{86}Sr ratios of soils and water reservoirs in the North-East Indian region also showed ^{87}Sr/^{86}Sr ratios in the range of 0.729 to 0.751.[29]

The agreement with the expected values of ^{87}Sr/^{86}Sr isotopic ratios was also seen for the tea samples (^{87}Sr/^{86}Sr, 0.714–0.723) produced in Munnar soils which are developed from sedimentary rocks in the peninsular region of the Western Ghats as well as the Sr isotopic composition of water reservoirs found in Munnar.[23] Finally, the tea samples analyzed from the Kangra region in this study gave ^{87}Sr/^{86}Sr values ranging from 0.719 to 0.724.

From Fig. 2b, it can be seen that Darjeeling tea samples show their distinctive ^{87}Sr/^{86}Sr ratio values as compared to those from Assam, Munnar and Kangra. But data on Assam region tea samples for ^{87}Sr/^{86}Sr are found to overlap with the Munnar and Kangra tea sample range. Although the ^{87}Sr/^{86}Sr ratio is considered to be one of the best geological tracers for food authentication in the literature,[4,11,14,15] in the present study, it was not possible to differentiate the four tea producing regions solely based on ^{87}Sr/^{86}Sr ratios.

3.4 Correlation between tea and soil ^{87}Sr/^{86}Sr ratios

As discussed in the literature, the ^{87}Sr/^{86}Sr isotopic ratio may vary with locations. Sr isotope abundance ratio values in tea plants could be related to their provenance soil due to the weathering of rocks, mobility of Sr to the soil–water system and subsequently to the parts of the plant. In this way, ^{87}Sr/^{86}Sr isotope ratios in tea leaves originating from different tea producing regions of India should be related to ^{87}Sr/^{86}Sr ratios in the soils.

Table 2 summarizes the observed range and mean ^{87}Sr/^{86}Sr ratios for soil samples and mean ^{87}Sr/^{86}Sr ratios of corresponding tea plants in brackets. Fig. 3 shows the correlation between the ^{87}Sr/^{86}Sr ratios of soil and corresponding tea plants from Assam, Darjeeling and Kangra. The correlation coefficient R^2 was >0.9 for soil–plant ^{87}Sr/^{86}Sr ratios. A statistical comparison through a paired *t*-test between values of ^{87}Sr/^{86}Sr ratios in tea plant leaves and soil also indicated that they do not differ significantly, which makes ^{87}Sr/^{86}Sr suitable for use as a tracer for the origin of tea samples.

3.5 Differentiation of Indian tea producing regions

Due to the overlapping patterns of isotopic composition data of Sr and C of tea samples, differentiation of four different tea producing regions could not be done unequivocally. Therefore, to achieve Indian tea origin differentiation in this study, combined data of the Sr content and isotopic composition of Sr and C of tea samples were used.[32]

Principal Component Analysis (PCA) was used as a pattern recognition method. PCA is an unsupervised data reduction method that calculates a new variable (principal or underlying components) as a linear combination of the original variable (score). Correlation coefficient between original variables and the new principal component indicates the weight of each single variable in the component, and is called component loading. PCA was performed on the combined data of the Sr content, ^{87}Sr/^{86}Sr ratios and δ^{13}C values of 38 tea (raw) samples

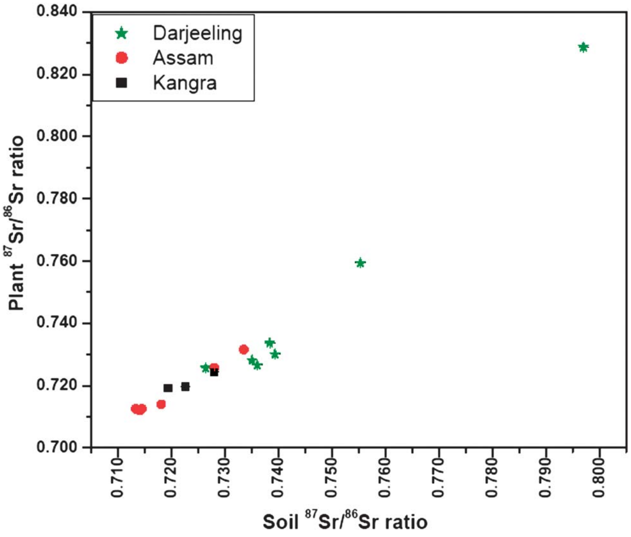

Fig. 3 Correlation between ^{87}Sr/^{86}Sr ratios of soil and the corresponding tea plants from Assam (●), Darjeeling (★), and Kangra (■).

using software package Matlab 7.5. PC1 and PC2 together were found to explain 71% of the total variance of the data. PC1 explained 41% of the total variance whereas PC2 explained 30% of the variance. The third PC3 component explained the remaining 29% of the variance. The combination of all PCs *i.e.* PC1, PC2 and PC3 gave a three dimensional plot which showed good differentiation among four tea producing regions as shown in Fig. 4. Munnar (Southern region) and Kangra (Northern region), which are two geographically well separated regions from Assam and Darjeeling (Northern-East), were found to be clearly differentiated. Since Assam and Darjeeling are the two geographically co-located nearby regions, their data are found to be close to each other as compared to Munnar and Kangra. This may be due to similar climatic conditions of these adjacent regions of India. Taking this factor into consideration, the use of stable isotope ratios might be improved by the use of climatic data as well as the multi-elemental profiling for these samples as additional variables, along with the study of factors affecting the isotopic composition of Sr and C, for the construction of a statistical model of differentiation with higher confidence.[22,27,28] It is also essential to broaden the database by analyzing a large number of tea leaves as well as soil samples from the four regions in India. This could allow the interpretation of results with enhanced confidence in the future. However, the study reported in this manuscript provides an insight and interesting observations to investigators working in this area.

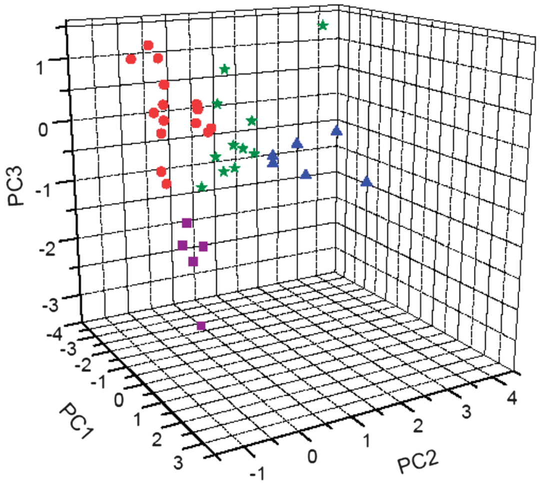

Fig. 4 Differentiation of Indian tea samples from Assam (●), Darjeeling (★), Munnar (▲) and Kangra (■) using PC1, PC2 and PC3 components of Principle Component Analysis.

4 Conclusions

In the present study, an analytical procedure based on the determination of ^{87}Sr/^{86}Sr and δ^{13}C in the tea samples by MC-ICP-MS and EA-IRMS, respectively, was developed and applied to 38 Indian tea samples obtained from different tea producing regions. Variations in ^{87}Sr/^{86}Sr ratios and δ^{13}C alone did not show differentiation among all regions. A well defined differentiation was observed among all the four tea producing regions on the basis of a combination of Sr content, Sr and C isotopic ratios data with multivariate analysis. The obtained data on ^{87}Sr/^{86}Sr ratios in authentic raw tea leaf samples can be useful while comparing them with the data from processed tea samples of respective regions.

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