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Application of Stable Isotope and Multi-Element Analysis in the Origin Traceability of Teak (*Tectona* spp.) Imported into China

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Abstract: Teak (*Tectona* spp.) is a precious species with high commercial value. Its characteristics vary from different growth regions, and its value will also be different. There has been a lack of reliable technological means to trace the origin of teak from various countries. In this study, we collected samples of teak trees in Myanmar, Cote d'Ivoire, and Panama from the ports where timber is imported into China to measure the content of 11 elements and the ratios of stable isotopes $\delta^{13}C$ and $\delta^{15}N$, which are used by inductively coupled plasma mass spectrometry (ICP-MS) and isotope ratio mass spectrometry (IRMS). Furthermore, software for statistical analysis, such as SPSS and SIMCA, was utilized to explore the statistical correlations of difference factors among samples from different countries and assess the application values of origin traceability. The results revealed significant differences ($p<0.05$) in the content of Rb, Sr, and Ba, as well as three rare earth elements (REEs)—La, Ce, and Nd—among the teak samples from the three countries. Moreover, a one-way analysis of variance (ANOVA) was performed on the $\delta^{13}C$ and $\delta^{15}N$ values, indicating significant differences ($p<0.05$) in $\delta^{13}C$ between the samples from Myanmar and those from Cote d'Ivoire and Panama. However, the $\delta^{13}C$ analysis alone was not effective in discriminating the samples from Cote d'Ivoire and Panama. In addition, an orthogonal partial least squares discriminant analysis (OPLS-DA) was conducted by combining isotope ratios and element content. This analysis resulted in an accuracy of 92.3% in discriminating between teak samples from the three countries, indicating a good level of discrimination. As such, it becomes feasible to achieve a certain degree of origin traceability for teak using $\delta^{13}C$ as the main difference factor for significance testing. The discriminant analysis combining isotope ratios and element content enhances the discrimination accuracy, which can be applied to trace the origin of teak imported from different regions.

Keywords: *Tectona* spp., Origin Traceability, Multi-Element, Stable Isotope Ratio, Import

1. Introduction

Due to a lack of domestic supply, China, a significant consumer of timber, is forced to rely substantially on timber imports every year in the global timber trade. The majority of these imports, namely logs and converted timber, come from several continents, including Africa, Southeast Asia, the Americas, and Europe [1, 2]. Among the types of timber that China imports, teak (*Tectona spp.*), a member of the family Verbenaceae, is trendy for high-end flooring, furniture, and interior decoration materials [3, 4]. Native to the tropics like Thailand, Myanmar, Indonesia, Ecuador, and Cote d'Ivoire,

teak is also planted in Yunnan and Guangdong provinces in China [5]. Nonetheless, there haven't been many reliable technological tools to trace the origin of teak from various countries. The differences in quality, cost, and trade measures between continents pose substantial challenges to the techniques for origin traceability in the timber import trade.

Traditional methods for identifying timber rely primarily on both macro-feature analysis and micro-anatomical analysis. However, these methods are limited to identifying timber species and can only determine the genus of timber, which cannot trace its origin [6]. To address this limitation, various techniques have emerged for the origin traceability of plants in

response to ever-growing needs. These techniques include gas chromatography-mass spectrometry (GC-MS) [7, 8], high-performance liquid chromatography (HPLC) [9], and ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) [10], which use specific organic substances in plants for identification. Inductively coupled plasma mass spectrometry (ICP-MS) is also used to compare and distinguish plants based on their elemental content differences [11]. Additionally, stable isotope ratio mass spectrometry (SIRMS) is particularly popular for analyzing the isotope fractionation patterns in different regions [12-15]. There have been several reports on studies using techniques in molecular biology for origin traceability, including the application of such techniques in tracing the origin of timber [16, 17]. Reports have also analyzed the advantages and limitations of DNA technology in the origin traceability of timber [18].

In terms of the origin traceability of timber, SIRMS commonly utilized for the identification using the isotope ratios of elements such as carbon, nitrogen, oxygen, and hydrogen [19, 20]. However, few studies have been conducted on using the analysis of both element content and stable isotope ratios to differentiate the origin of timber from various countries.

2. Materials and Methods

2.1. Samples

Wood samples were collected from Ningbo port (Zhejiang Province, China) and Huangpu Port (Guangdong Province, China) respectively, including 16 samples from Myanmar (Asia), 6 samples from Cote d'Ivoire (Africa), and 4 samples from Panama (Central and South America).

2.2. Reagents

The standard solutions (separately 1000 μg/ml) of magnesium, manganese, iron, copper, zinc, rubidium, strontium, barium, lanthanum, cerium, neodymium, and internal standard solutions (100 μg/ml) of scandium, germanium, yttrium, indium, terbium, were purchased from National Analysis and Testing Center for Nonferrous Metals and Electronic Materials (Beijing, China). Nitric acid (electronic grade) was purchased from Suzhou Jingrui Chemical Co., Ltd. (Suzhou, China). Powdered copper and tungsten trioxide (WO_3) (analytical reagent) were purchased from Elementar (Germany). Reference materials used in isotope ratio tests including B2155 (δ^{13}C: -26.98‰, δ^{15}N: 5.94‰), USGS40 (δ^{15}N: -4.52‰), IAEA-CH1-4 (δ^{13}C: -10.45‰), USGS 64 (δ^{13}C: -40.82‰), IAEA-N-2 (δ^{15}N: 20.3‰) were provided by Elementar (Germany). The experimental ultra-pure water was provided by ultra-purified water system.

2.3. Apparatus

CPA224S electronic balance (accuracy 0.1 mg, Sartorius, Germany); NexION 350X inductively coupled Plasma Mass Spectrometer (PerkinElmer Instruments, USA); CEM Mars 5 microwave digester (CEM Corporation, USA)); Isotope cube (Elementar, Germany); Isotope Ratio Mass Spectrometer (Biovision, Elementar, Germany).

2.4. Methods

2.4.1. Sample Treatment

The method for determining elements involved cutting the teak sample into specific chips and then into small particles. A precise amount of 0.2 g was weighed and placed in a digestion vessel. Concentrated nitric acid (5 ml) was added, and the vessel was immediately sealed. The vessel was then placed in the microwave digestion apparatus under the temperature control mode. The temperature was initially set to 120°C for 5 minutes, then raised to 140°C and maintained for 20 minutes. After cooling, the digestion vessel was opened for acid evaporation. The solution was then filtered and adjusted to a volume of 200 ml. Finally, the elements were determined through ICP-MS, with Sc, Ge, Y, In, and Tb used as internal standard solutions for quantification.

The method used to determine the ratios of stable isotopes δ^{13}C and δ^{15}N involved grinding the teak sample into powder. A small amount (approximately 20 mg) of the sample was placed in a tin capsule and inserted into the sample tray of an elemental analyzer. In the elemental analyzer, the carbon and nitrogen elements in the sample were converted into CO_2 and N_2, respectively, and then measured for δ^{13}C and δ^{15}N through SIRMS. The combustion tube of the elemental analyzer contained WO_3 as the main filler, which was heated to 1150°C. The reduction tube, which contained Cu as the filler, was heated to 850°C. Additionally, high-purity helium (He, 99.999%) was used as the carrier gas.

The formula for calculating the ratios of stable isotopes δ^{13}C and δ^{15}N is:

$$\delta E = (R_{sample} - R_{standard}) / R_{standard} \qquad (1)$$

Where E is the isotope of the element being studied, R_{sample} is the abundance ratio of heavy isotope to light isotope in the sample measured, such as ^{13}C/^{12}C and ^{15}N/^{14}N, and $R_{standard}$ is the abundance ratio of heavy isotope to light isotope in the standard sample. The δ value, when multiplied by 1,000, gave the final unit in ‰. The results were calibrated using international reference materials (VPDB for δ^{13}C and AIR for δ^{15}N).

2.4.2. Data Processing

For data processing, the results underwent statistical significance analysis using IBM SPSS Statistics 26.0, and a box plot was generated. Furthermore, the results were also analyzed through OPLS-DA using Umetrics SIMCA 14.1, and an OPLS-DA 2D score plot was produced.

3. Analysis and Discussion

3.1. Analysis of Multi Elements

This paper investigates the content of 11 elements (i.e. Mg, Mn, Fe, Cu, Zn, Rb, Sr, Ba, La, Ce, and Nd) in teak samples from three countries. Some of them are essential for the growth of trees as they provide necessary nutrients, such as Mg, Mn, Fe,

Cu, and Zn. In addition, rare earth elements (REEs), including La, Ce, and Nd, were also examined. ICP-MS was used to measure the content of these elements, and the results are shown in Table 1. According to the table, Mg was the element in the highest content, with an average of 903 mg/kg. It was significantly higher compared to the other elements in the teak samples, being 12.9 times higher than the second-highest element, Fe. The content of the three REEs, by contrast, were typically below 0.3 mg/kg on average. Moreover, significant differences were observed in the content of these three elements among the samples from the three countries. The samples from Panama, in particular, exhibited significantly lower content of these three elements compared to those from the other two countries. The Kruskal-Wallis Test (H Test) was carried out to further assess these 11 elements. This statistical analysis revealed significant differences ($p<0.05$) in the content of Rb, Sr, Ba, La, Ce, and Nd among the three groups of samples. The significance level of less than 0.05 indicates that these six elements exhibit significant differences among the samples from teak trees in the three countries.

Table 1. *Analysis results of element concentration of Tectona spp. timber from different countries.*

Element	Average concentration (mg/kg)			Standard deviation			Significant Difference (P)
	Myanmar	Cote d'Ivoire	Panama	Myanmar	Cote d'Ivoire	Panama	
Mg	827	916	1132	390	283	232	0.230
Mn	3.89	3.89	4.63	0.948	0.908	1.34	0.562
Fe	69.0	56.3	54.4	33.1	11.2	5.04	0.872
Cu	10.1	11.0	11.8	4.32	3.28	2.42	0.327
Zn	19.1	14.8	18.1	10.0	7.38	4.91	0.540
Rb	0.286	0.732	0.941	0.288	0.563	0.480	0.032
Sr	7.15	12.0	9.22	2.93	1.94	1.78	0.011
Ba	11.4	16.1	10.2	8.88	4.56	1.33	0.035
La	0.187	0.306	0.010	0.169	0.318	0.004	0.007
Ce	0.102	0.188	0.016	0.071	0.217	0.004	0.009
Nd	0.080	0.137	0.007	0.064	0.163	0.003	0.008

3.2. Analysis of Stable Isotope Ratios

Table 2 displays the analysis results for $\delta^{13}C$ and $\delta^{15}N$ in samples from the three different countries. The samples from Myanmar, Cote d'Ivoire, and Panama had standard deviations (SDs) for $\delta^{13}C$ of 0.490, 0.334 and 0.237, respectively. These values were respectively 0.75, 0.14, and 0.32 times the standard deviations (SDs) for $\delta^{15}N$ in the corresponding country samples. These results suggest that $\delta^{13}C$ exhibits less dispersion and greater stability. Among the three groups of samples, the maximum RSD for $\delta^{13}C$ was 2.32%, which was significantly lower than those for the other elements and $\delta^{15}N$. This difference may be attributed to the fact that the carbon isotope abundance variation in teak trees primarily originates from atmospheric CO_2 in its place of origin, whereas the nitrogen isotope abundance variation, in addition to being sourced from the atmosphere of its origin, is more influenced by soil factors such as animal wastes and fertilizers.

Table 2. *Stable isotope ratio and statistical analysis results of Tectona spp. timber from different countries.*

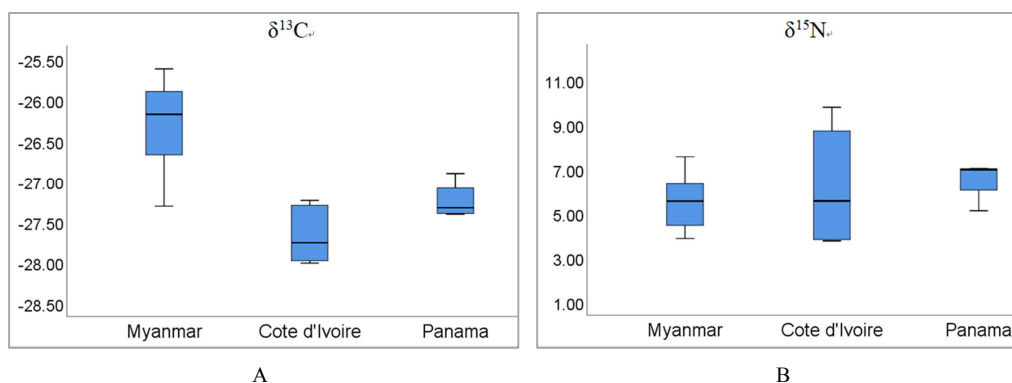| Statistical result		$\delta^{13}C$			$\delta^{15}N$		
		Myanmar	Cote d'Ivoire	Panama	Myanmar	Cote d'Ivoire	Panama
Mean (‰)		-26.3	-27.7	-27.2	5.49	6.19	6.54
Standard deviation		0.490	0.334	0.237	1.14	2.71	0.96
RSD (%)		1.99	2.32	0.868	20.8	43.7	14.7
95% confidence	lower limit	-26.5	-28.0	-27.6	4.88	3.34	5.01
interval of the mean	upper limit	-26.0	-27.3	-26.9	6.10	9.04	8.06
F Value		25.862			0.894		
P		0.000			0.423		

Figure 1. *$\delta^{13}C$(A) and $\delta^{15}N$(B) box plot of Tectona spp. timber from different countries.*

The $\delta^{13}C$ and $\delta^{15}N$ values were subjected to a one-way analysis of variance (ANOVA) using SPSS. The results indicated significant differences in the $\delta^{13}C$ values among the teak samples from the three countries, with a p-value of 0.000 (p< 0.05). The post-hoc test for multiple comparison analysis, however, was carried out to investigate whether there were significant differences between each pair of countries. The $\delta^{15}N$ values, on the other hand, showed overlap yet no significant difference.

Table 3. *The significance of multiple post-hoc comparative analysis of $\delta^{13}C$ and $\delta^{15}N$ in Tectona spp. timber from different countries.*

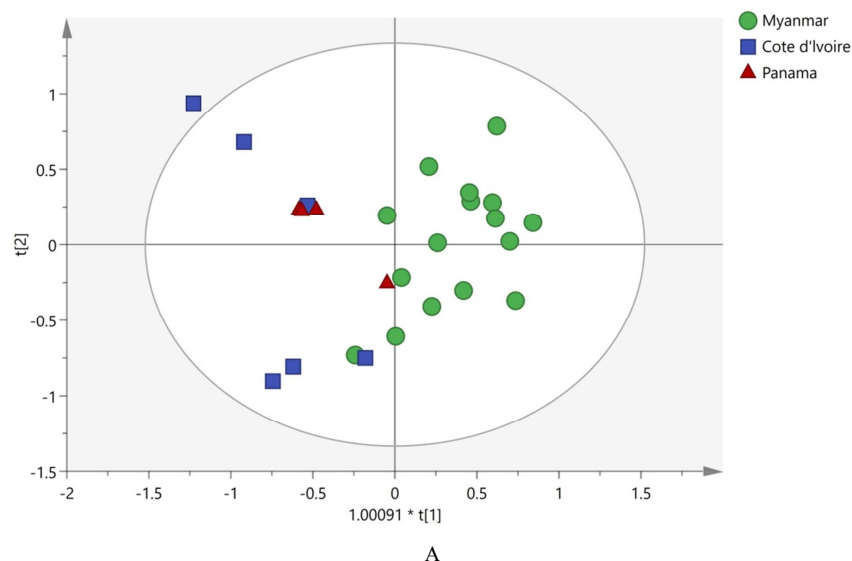| Dependent Variable	Origin Country (I)	Origin Country (J)	P
$\delta^{13}C$	Myanmar	Cote d'Ivoire	0
		Panama	0.001
	Cote d'Ivoire	Myanmar	0
		Panama	0.136
$\delta^{15}N$	Myanmar	Cote d'Ivoire	0.372
		Panama	0.256
	Cote d'Ivoire	Myanmar	0.253
		Panama	0.795

The post-hoc test for multiple pairwise comparison analysis was performed using SPSS to examine the $\delta^{13}C$ and $\delta^{15}N$ values in the teak samples from the three countries. The results, which can be referred to in Table 3, revealed significant differences (p<0.05) in $\delta^{13}C$ between the samples from Myanmar and those from Cote d'Ivoire and Panama. However, no significant difference in $\delta^{13}C$ was observed between the samples from Cote d'Ivoire and Panama. To visually represent the ratios of stable isotopes $\delta^{13}C$ and $\delta^{15}N$ in these samples, a box plot was created. In Figure 1, the categorical axis denotes the importing countries, while the vertical axis represents SIRMS. Notably, the samples from Myanmar exhibited the highest mean value of $\delta^{13}C$, whereas the samples from Cote d'Ivoire displayed the lowest mean value. To further analyze the mean values of $\delta^{13}C$, the 95% confidence interval was examined, as depicted in Table 2. The lower limit value of $\delta^{13}C$ in the samples from Myanmar was -26.5‰, whereas the range of values in the samples from Panama was from -27.6‰

to -26.9‰. In the samples from Cote d'Ivoire, the upper limit value was -27.3‰. The $\delta^{13}C$ range in the samples from Myanmar significantly differed from the ranges observed in the samples from the other two countries. The $\delta^{13}C$ ranges in the samples from Panama and Côte d'Ivoire, however, partially overlapped, showing less discrimination. It is obvious to note that the wide range of $\delta^{15}N$ in the samples from Cote d'Ivoire affected the difference analysis.

3.3. Discriminant Analysis of Isotope Ratios and Element Content

This paper focuses on analyzing samples from teak trees in Asia, Africa, and Central America. It is well established that these trees experience varying growth conditions across different countries. These variations can cause differences in the abundance of isotopes and content of elements, which, in turn, can be utilized to determine the origin of timber. However, several factors can affect the isotopic and elemental characteristics of timber, such as the soil, atmosphere, and surrounding organisms. This can pose a challenge when attempting to accurately discriminate between the characteristic factors of timber from different countries. By comparing isotope ratios, it has been observed that while the $\delta^{13}C$ values can successfully discriminate teak samples from Myanmar from those in the other two countries, they are not effective in tracing the origin of teak from Côte d'Ivoire and Panama.

Additionally, a comprehensive analysis through OPLS-DA was performed on the data. Initially, the $\delta^{13}C$ and $\delta^{15}N$ values of the teak samples from the three countries were analyzed using OPLS-DA. This analysis allowed us to create a score plot, as shown in Figure 2A. According to the plot, the samples from Myanmar are mainly concentrated in the first and fourth quadrants, which are distinguishable from the samples from Cote d'Ivoire and Panama based on the y-axis. The samples from Cote d'Ivoire are primarily found in the second and third quadrants and show dispersion, with some overlap with the samples from Panama. The accuracy of discriminating the samples from the three countries was 80.8%.
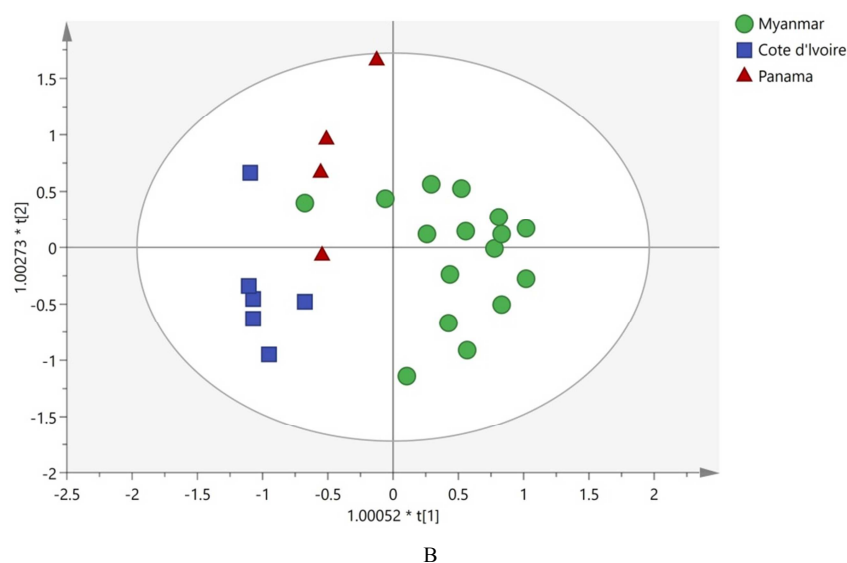

A

B

Figure 2. *OPLS-DA 2D score plot of $\delta^{13}C$, $\delta^{15}N$ value (A) and Comprehensive data (B) in timber samples from three countries.*

To enhance the accuracy in determining the origin of the samples, this paper conducted an analysis using a combination of $\delta^{13}C$, $\delta^{15}N$, and 11 elements. The min-max scaling on the data of $\delta^{13}C$ and $\delta^{15}N$, as well as Sr, Rb, La, Ce, Nd etc., in combination with the use of OPLS-DA, resulted in a discrimination accuracy of 92.3% and a score plot, as shown in Figure 2B, which demonstrates enhanced aggregation within groups and better separation between groups compared to Figure 2A. In terms of sample discrimination, there were two misclassifications. One sample from Myanmar was mistakenly classified as Cote d'Ivoire, and another sample from Panama was also misclassified as Cote d'Ivoire. After 200 permutation tests performed on the discriminative model, the validation of the model can be considered successful. As such, this model successfully achieved accurate traceability of the origin of samples from teak trees in Myanmar, Cote d'Ivoire, and Panama.

4. Conclusion

This paper focuses on samples from teak trees in Myanmar, Cote d'Ivoire, and Panama to measure and analyze the content of 11 elements and the ratios of stable isotopes $\delta^{13}C$ and $\delta^{15}N$ using SPSS and SIMCA. The results revealed significant differences (p<0.05) in the content of Rb, Sr, Ba, and three REEs among the samples from the three countries. The RSD of $\delta^{13}C$ in samples from different origins was less than or equal to 2.32%, which was significantly lower than those observed in $\delta^{15}N$ and other elements. A one-way ANOVA for the difference test confirmed significant differences (p<0.001) in $\delta^{13}C$ among the samples from the three countries. Additionally, the post-hoc test for multiple pairwise comparison analysis showed significant differences (p<0.05) in $\delta^{13}C$ between the samples from Myanmar and those from Cote d'Ivoire and Panama, while there was some overlap in $\delta^{13}C$ between the samples from Cote d'Ivoire and Panama. The min-max scaling on the data of $\delta^{13}C$ and $\delta^{15}N$, as well as Sr, Rb, La,

Ce, Nd etc., in combination with the use of OPLS-DA, achieved a discrimination accuracy of 92.3% for the teak samples from the three countries. This represents an improvement of 11.5% compared to using isotope ratios alone for discriminant analysis, thereby allowing for more accurate discrimination. The analytical techniques and statistical methods employed in this paper effectively trace the origin of samples from teak trees in the three countries. Further research on this methodology has the potential to yield even better discrimination results. In conclusion, the significant differences in $\delta^{13}C$, coupled with the discriminant analysis of isotope ratios and multi elements, assure traceability of the origin of teak from different countries, such as Myanmar, Cote d'Ivoire, and Panama. Moreover, this methodology is highly valuable for timber importing countries, as it provides technical support for tracing the origin of timber imports and exports and verifying the legality of timber origins.

Acknowledgements

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