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Research Article

Identifying the Geographical Origin of Tobacco Leaf by Strontium and Lead Isotopic with Mineral Elemental Fingerprint

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The primary aim of this paper was to identifying the geographical origin of tobacco leaves based on stable isotopic and mineral elemental fingerprint. We collected eighty-one tobacco leaf samples from Argentina, Brazil, Zimbabwe, the U.S., Zambia, and China. And nine mineral element contents and four strontium and lead isotope ratios of the tobacco leaves were determined by thermal ionization mass spectrometry (TIMS) and inductively coupled plasma mass spectrometry (ICP-MS). After variance and stepwise discriminant analysis, the discriminant functions of the tobacco leaf's geographical origin were established. The results indicate that: (1) the contents of six mineral elements including Cu, Zn, Cr, Ni, Cd, and Pb, together with four strontium and lead isotope ratios containing $^{87}Sr/^{86}Sr$, $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$, were significantly different among six countries. (2) Different countries presented some characteristic mineral elemental and isotopic fingerprint. The even contents of mineral elements from Zambian tobacco leaf were much lower than the other countries, among which four elements consisting of Zn, Cr, As, and Cd were not detected. The three average lead isotope ratios including $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$ from Zimbabwe tobacco leaves were far higher than the other countries, and the range of which was unoverlapped. (3) The effective identification of the geographical origin of tobacco leaf was accomplished by Fisher stepwise discriminant analysis and the characteristic tracing elements consisted of Cu, Zn, Cr, Ni, Cd, Pb, $^{87}Sr/^{86}Sr$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$. Based on the established discriminant functions, the original and cross-validation accuracy towards different geographical origins of tobacco leaves were 98.8% and 95.1%, respectively. The study shows that the strontium and lead isotopic with mineral elemental fingerprints is a potential effective method to identify the geographical origin of tobacco leaves from different countries.

1. Introduction

With the globalization of the economy, agricultural products' transnational and cross-regional circulation are increasingly frequent, and the quality and safety of agricultural products have become a common concern of the world today [1, 2]. The geographical origin of agricultural products is one of the important quality and safety elements. Even more, some laws and regulations expressly require the labeling of the geographical origin. The European Union regulations allow the application of the following geographical indications to a food product: protected designation of origin (PDO), protected geographical indication (PGI), and traditional specialties guaranteed (TSG) [3, 4].

Many studies have been conducted to investigate the geographical origin of agricultural products, mainly through DNA, near-infrared spectroscopy, stable isotopes, mineral elements, feature organic content, etc., to find specific indicators that can characterize regional information [3, 4]. Among them, stable isotope fingerprints are a natural label of crops, closely related to their growing environment and location, and do not change in the process. They can provide independent and unchangeable identification information

for its origin traceability. Therefore, stable isotope detection technology combined with mineral element content analysis is the most effective method to trace the origin of agricultural products [5]. At present, this technology has been widely used in the origin traceability of wine [6], beer [7], spring water [8], tea [9], coffee [10], rice [11], lamb [12], beef [13], poultry meat [14], edible vinegar [15], wheat [16], and cocoa [17]. It has provided strong technical support for the protection of geographical indications of world-renowned agricultural products and processed products.

Tobacco leaves are important economic crops in the world, and their quality is closely related to genetic factors, cultivation measures, modulation techniques, origin soil and climatic conditions, etc., among which the effect of the production areas such as soil and climatic conditions on the quality of tobacco leaves is highly significant [18]. Due to the high taxes and fees on tobacco, smuggling and illegal sales are very active. These activities violate legal regulations and lead to severe financial and tax losses. Taking the European Union as an example, illegal sales cause tax and customs losses of 10 billion every year. Tobacco leaves are the foundation of the cigarette industry. The quality and authenticity of the origin directly affect the quality and authenticity of cigarettes. Therefore, the unauthentic origin of tobacco leaves is one of the primary forms of cigarette counterfeiting [19].

In the world, China is the world's largest producer and importer of tobacco leaves, while Zimbabwe, Brazil, and the United States are the world's largest tobacco leaf exporters. In international trade, counterfeit tobacco often appears, which seriously affects the financial revenue of the government and the legitimate rights and interests of consumers.

There are many researches about tobacco origin tracing, mainly based on volatile components of tobacco leaves [20], metabolites [21], nitrogen-containing compounds [22], pollen content [23], X-ray fluorescence [24], near-infrared spectroscopy [25], and color fractal [26], but these methods are easily affected by factors such as fertilization type, redrying process, picking period, and storage conditions. Isotope ratio characteristics generally do not change due to chemical and physical changes experienced, and they are an effective indicator for judging the origin of animal and plant products [27]. Because of this, many researchers use stable isotope technology to carry out exploratory research on the traceability of tobacco leaves or cigarettes. For example, the position specific isotope analysis (PSIA) was used to study the intrinsic relationship between the hydrogen isotope ratio in nicotine and the origin of tobacco leaves [28], and/or use the compound specific isotope analysis (CSIA) to explore the ratio of carbon, hydrogen, and oxygen isotopes in nicotine to trace the tobacco [29], and/or by bulk stable isotope analysis (BSIA) to study lead isotope ratio to trace tobacco in different provinces of China [30].

The aims of this study were to develop reliable analysis technology that could establish the geographical origin of tobacco leaves based on their trace mineral elemental "fingerprint" and the stable isotope ratios of strontium and lead. Here we present the findings from tobacco samples originating from Argentina, Brazil, Zimbabwe, the U.S., Zambia, and China.

2. Materials and Methods

2.1. Sample Collection and Preparation. The 81 tobacco leaf samples were primary-cured tobacco and red-cured sheet tobacco collected from 6 different countries in 2013–2014, including 12 from Argentina, 10 from Brazil, 17 from Zimbabwe, 6 from the United States, 8 from Zambia, and 28 from China.

Samples thus prepared were ground to obtain the powder by a Cyclotec sample mill (SMF2002, SUPOR, China).

2.2. Elemental Analysis. The elemental analysis is based on YC/T 380–2010 "Tobacco and Tobacco products chrome, nickel, arsenic, selenium, cadmium, lead assay inductive coupling plasma mass spectrometry" [31].

A microwave digestion system (MW3000, Anton Paar, Austria) was used to digest the pretreated tobacco samples, and the digestion process was as follows: a certain amount of samples (0.2~0.3 g) and 65% HNO_3 solution (5 mL, BV-III, Beijing Institute of Chemical Reagents) and 30% H_2O_2 solution (2 mL, BV-III, Beijing Institute of Chemical Reagents) were added into a Teflon digestion vessel for digestion. In the 60-min digestion process, the temperature gradually rose to 190°C. Finally, the digested liquid was diluted with ultrapure water to 50 mL and stored in a plastic storage bottle before analysis.

The concentrations of nine elements (Cu, Zn, Co, Cr, Ni, As, Se, Cd, and Pb) were determined by inductively coupled plasma mass spectrometry (ICP-MS, ELAN-DRC-e, PerkinElmer, USA).

2.3. Sr and Pb Isotope Analysis. The measurement of the isotopic ratio of the Sr and Pb is based on GB/T 17672–1999 "Determinations for isotopes of lead, strontium, and neodymium in rock samples" [32]. Pretreatment method: 0.5 g powder was weighed into a quartz beaker, and 65% HNO_3 solution was added to dissolve it overnight. It was dried and then heated at 250°C for 2 h and at 550°C for 4 h in a muffle furnace, after that 1 ml 65% HNO_3 was added to dissolve and then it was transferred to the Teflon vessel and finally evaporated to be tested. Separation and purification of Sr and Pb are completed using the AG50W-X8 cation exchange resin (Pharmaceutical Group Chemical Reagent Co., Ltd. China) and the AG1-X8 anion exchange resin (Bio-Rad Laboratories, UK).

The Sr and Pb isotope ratios ($^{87}Sr/^{86}Sr$, $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$) were measured by thermal ionization mass spectrometry (TIMS, PHOENIX, Isotopx, UK).

2.4. Statistics. The statistical analysis of the data was used by SPSS 22.0 software (IBM, US).

The analysis of variance (ANOVA) was carried out for each element. Firstly, the variance homogeneity test for each variable was carried out to check whether the total variances of each analytical index groups were consistent. Then, at the

Table 1: Mineral element content in the tobacco leaf from different countries (mg/kg).

nation	Statistical indicator	Cu	Zn	Co	Cr	Ni	As	Se	Cd	Pb
Argentina (n = 12)	Ave.	4.64^{c}	10.31^{d}	0.32^{bc}	0.83^{a}	2.05^{b}	0.31^{ab}	0.12^{b}	0.24^{b}	0.84^{b}
	S.D.	1.34	1.69	0.08	0.26	0.36	0.10	0.05	0.20	1.42
	Min.	2.94	8.06	0.21	0.45	1.36	0.20	0.05	0.05	0.00
	Max.	7.74	13.28	0.43	1.28	2.71	0.52	0.19	0.83	4.78
Brazil (n = 10)	Ave.	6.03^{bc}	18.84^{c}	0.41^{b}	0.29^{c}	0.97^{bc}	0.08^{b}	0.09^{b}	0.39^{b}	0.09^{c}
	S.D.	1.35	4.00	0.09	0.08	0.28	0.04	0.04	0.12	0.29
	Min.	3.93	13.42	0.28	0.21	0.61	0.04	0.05	0.22	0.00
	Max.	7.82	24.80	0.58	0.43	1.57	0.17	0.17	0.56	0.92
Zimbabwe (n = 17)	Ave.	10.52^{a}	28.64^{b}	0.65^{a}	0.73^{ab}	2.14^{b}	0.11^{b}	0.25^{a}	0.10^{b}	0.70^{bc}
	S.D.	2.62	5.16	0.27	0.23	0.52	0.05	0.12	0.04	0.57
	Min.	7.23	22.35	0.38	0.36	1.42	0.03	0.11	0.00	0.00
	Max.	16.01	44.20	1.59	1.12	3.08	0.22	0.52	0.15	1.61
United States (n = 6)	Ave.	8.50^{ab}	40.15^{a}	0.14^{c}	0.69^{ab}	0.78^{c}	0.11^{b}	0.14^{b}	0.50^{b}	0.30^{bc}
	S.D.	1.83	1.60	0.09	0.34	0.47	0.06	0.08	0.33	0.27
	Min.	5.24	38.25	0.05	0.36	0.23	0.03	0.05	0.08	0.12
	Max.	10.16	42.37	0.31	1.21	1.31	0.19	0.26	0.78	0.84
Zambia (n = 8)	Ave.	0.79^{d}	0.00^{e}	0.36^{bc}	0.00^{d}	0.24^{c}	0.00^{b}	0.19^{ab}	0.00^{b}	0.10^{c}
	S.D.	1.47	0.00	0.09	0.00	0.22	0.00	0.11	0.00	0.08
	Min.	0.00	0.00	0.26	0.00	0.00	0.00	0.06	0.00	0.00
	Max.	3.53	0.00	0.55	0.00	0.53	0.00	0.36	0.00	0.25
China (n = 28)	Ave.	10.25^{a}	34.27^{ab}	0.53^{ab}	0.58^{b}	5.27^{a}	0.75^{a}	0.25^{a}	2.91^{a}	2.60^{a}
	S.D.	5.42	18.75	0.46	0.54	3.25	1.40	0.21	1.83	1.13
	Min.	2.11	3.16	0.11	0.12	1.95	0.11	0.01	0.64	0.92
	Max.	22.35	77.44	2.12	2.10	15.19	7.68	0.80	8.95	6.08
Total (n = 81)	Ave.	7.89	24.69	0.46	0.56	2.78	0.35	0.19	1.15	1.21
	S.D.	4.76	16.61	0.33	0.43	2.71	0.87	0.15	1.68	1.37
	Min.	0.00	0.00	0.05	0.00	0.00	0.00	0.01	0.00	0.00
	Max.	22.35	77.44	2.12	2.10	15.19	7.68	0.80	8.95	6.08
Significant difference between regions difference	p value	0.000	0.000	0.006	0.000	0.000	0.069	0.012	0.000	0.000
	F value	13.003	17.759	3.569	7.029	15.080	2.144	3.171	22.032	20.795

*Different uppercase characters in each column indicate significantly different mean values according to Duncan test ($p < 0.05$).

significance level $p < 0.05$, Duncan's multiple comparison was performed to determine the significant difference between the individual regions when the F value was significant in ANOVA.

The Fisher stepwise discriminant analysis is employed to classify the geographical origin. The robustness of the classification model was evaluated by the original validation and cross-validation tests.

3. Results and Discussion

3.1. Difference Analysis of Mineral Element Content in Tobacco from Different Countries. The content of 9 mineral elements in tobacco leaves from Argentina, Brazil, Zimbabwe, the United States, Zambia, and China was analyzed by ANOVA. The measurement and statistical results are shown in Table 1, Figure 1–9. The contents of Cu, Zn, Ni, Cd, and Pb are relatively higher than the other minerals in all tobacco leaf samples with average values of 7.89, 24.69, 2.78, 1.15, and 1.21 mg/kg, and the contents of Co, Cr, As, and Se have relatively lower average, with values of 0.46, 0.56, 0.35, and 0.19 mg/kg, respectively. Among them, the element contents of Cu, Zn, Cr, Ni, Cd, and Pb were significantly different among the 6 countries ($p < 0.001$, F value ˃7). In contrast, the element contents of Co, As and Se were not significantly different ($p > 0.001$).

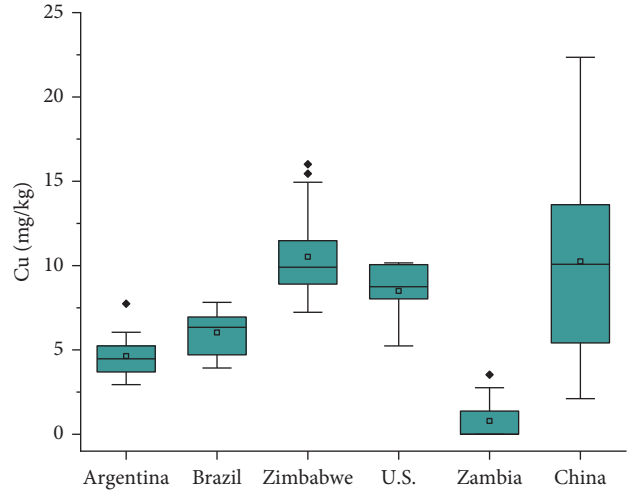

Figure 1: The box plots of Cu content.

The contents of mineral elements in tobacco leaf samples from different countries have their own characteristics. The content of Cr in Argentine tobacco leaves is significantly

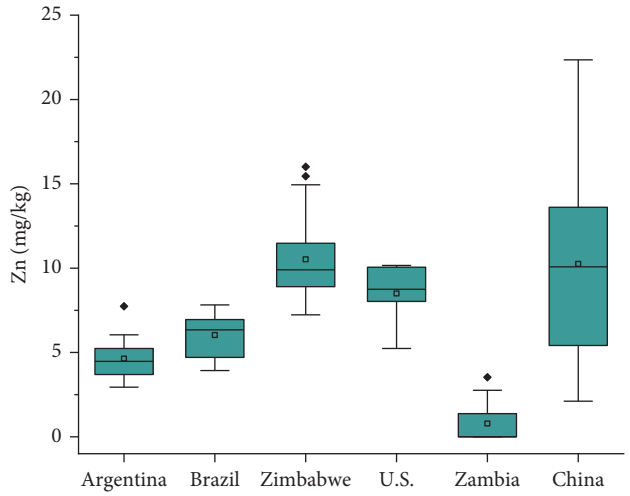

Figure 2: The box plots of Zn content in tobacco leaf from different countries in tobacco leaf from different countries.

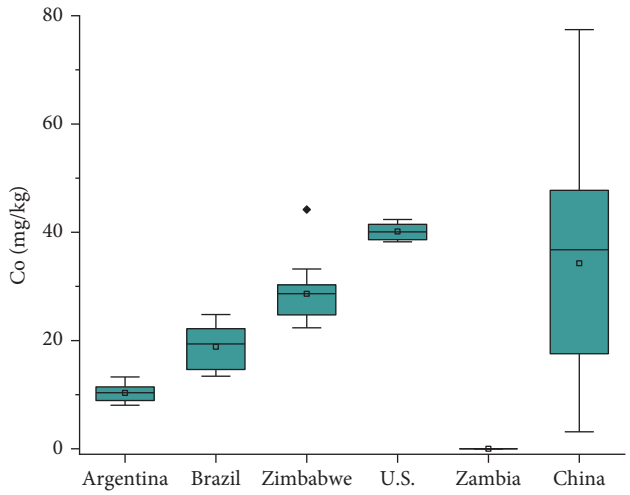

Figure 3: The box plots of Co content.

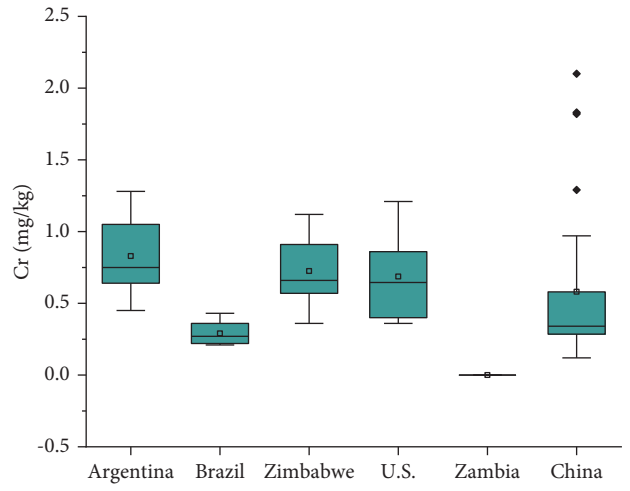

Figure 4: The box plots of Cr content in tobacco leaf from different countries in tobacco leaf from different countries.

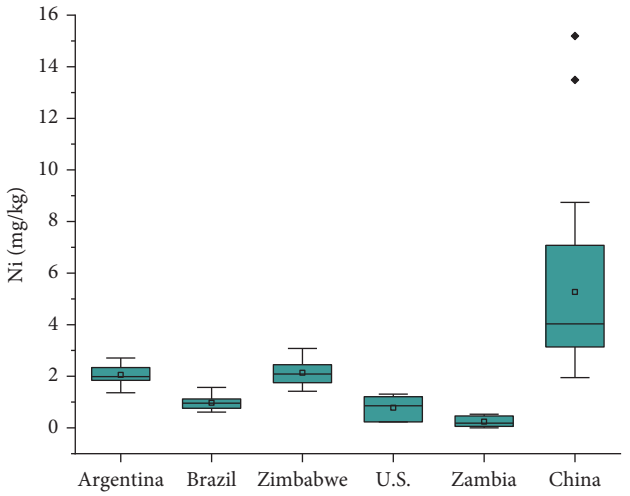

Figure 5: The box plots of Ni content.

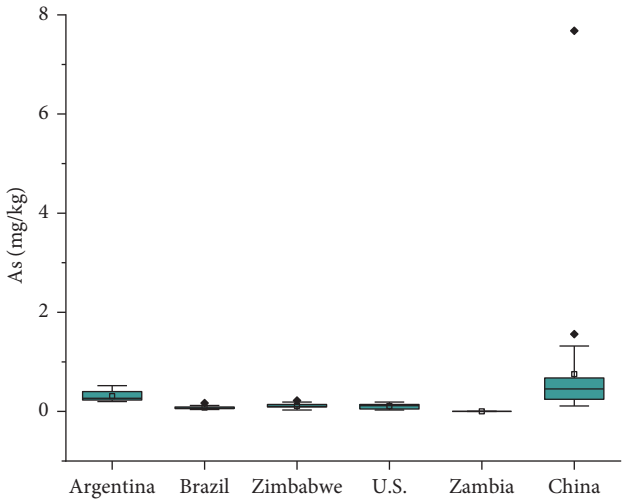

Figure 6: The box plots of As content in tobacco leaf from different countries in tobacco leaf from different countries.

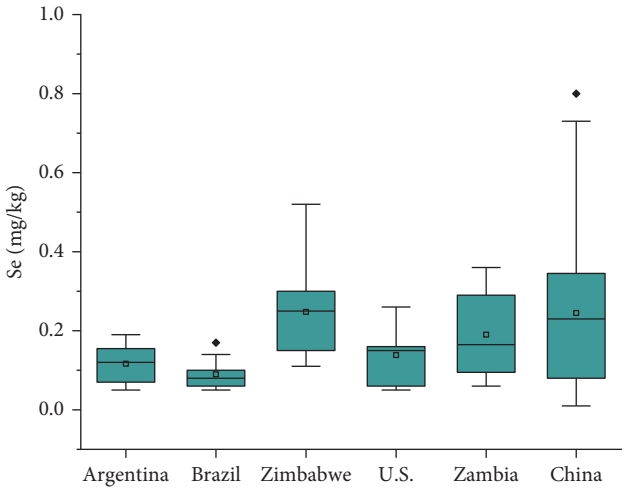

Figure 7: The box plots of Se content.

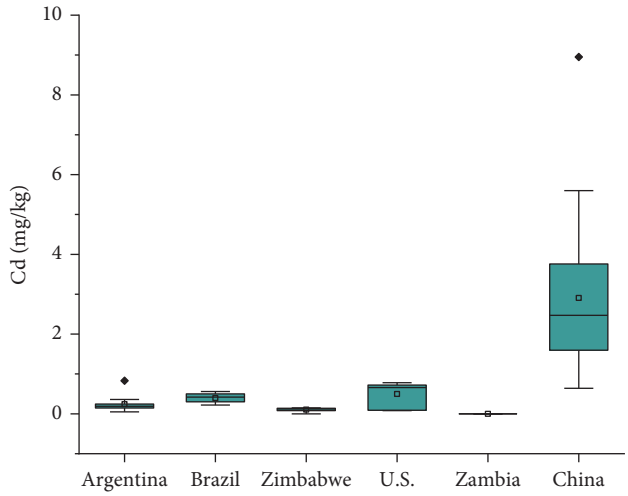

Figure 8: The box plots of Cd content in tobacco leaf from different countries in tobacco leaf from different countries.

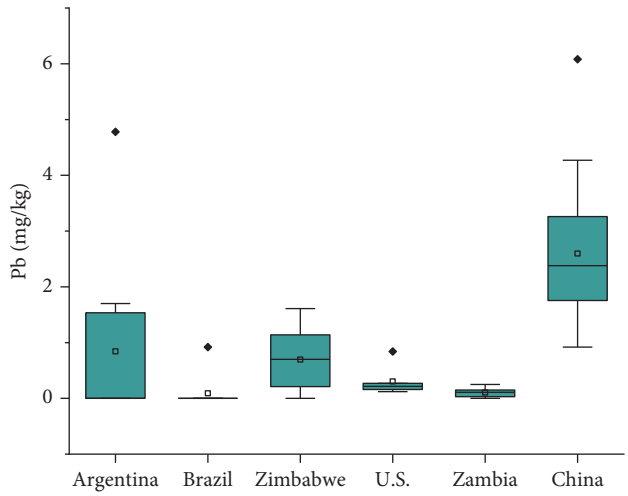

Figure 9: The box plots of Pb content in tobacco leaf from different countries.

higher than that of the other 5 countries, with an average of 0.83 mg/kg. The content of Pb in Brazilian tobacco leaves is lower than that of the other 5 countries, with an average of 0.09 mg/kg, and the content of other elements is in medium level. The content of Cu, Co, and Se in Zimbabwe tobacco leaves was significantly higher than those of the other 5 countries, with the average values of 10.52, 0.65, and 0.25 mg/kg, respectively. The content of Zn in American tobacco leaves was the highest, with an average value of 40.15 mg/kg, and the content of Co was the lowest, with an average value of 0.14 mg. It is worth noting that the content of mineral elements in tobacco leaves in Zambia is generally low, and the content of Cu and Ni is significantly lower than that of the other 5 countries, with an average of 0.79 and 0.24 mg/kg, respectively; At the same time, Zn, Cr, As, and Cd are not detected. The contents of Ni, As, Se, Cd, and Pb in tobacco leaves in China were significantly higher than those in the other five countries, with average values of 5.27, 0.75, 0.25, 2.91, and 2.60 mg/kg, respectively.

3.2. Characteristics of Sr and Pb Isotopes in Tobacco from Different Countries. The results of 4 isotopic ratio measurements in different countries are shown in Table 2, and the box plots are shown in Figure 10–13. The difference between the isotopic ratio between the six countries was significant ($p < 0.001$, $F > 116$). The Sr and Pb isotope ratio of Zimbabwe tobacco leaves has obvious characteristics. The average values of $^{87}Sr/^{86}Sr$, $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$ are significantly higher than those of the other five countries, which are 0.74148, 41.122, 16.450, and 22.388, respectively. Except for the overlap of $^{87}Sr/^{86}Sr$ with Zambia, $^{208}Pb/204Pb$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$ have no overlap at all, and these ratios can be easily distinguished from other countries by comprehensive use of these ratio characteristics. Among the remaining 5 countries, the $^{87}Sr/^{86}Sr$ of Zambian tobacco is the highest, with an average value of 0.73999, which can be well differentiated from the other 4 countries. The values of $^{87}Sr/^{86}Sr$, $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$ have partially overlapped with Brazil, the United States, and China, but have no overlap with Argentina at all. And among the four countries of Argentina, Brazil, the United States and China, the four Sr and Pb isotope ratios all overlap. The maximum value of $^{206}Pb/^{204}Pb$ in Argentine tobacco leaves is 18.537, and the minimum values of $^{206}Pb/^{204}Pb$ in Brazilian and American tobacco leaves are 18.550 and 18.560, respectively. Therefore, $^{206}Pb/^{204}Pb$ can better identify Argentine, Brazilian, and American tobacco leaves.

3.3. Traceability of Tobacco Origin. From the above analysis, it can be seen that the fingerprints of mineral elements, Sr and Pb isotope ratios in tobacco leaves in 6 countries all show specific characteristics. To further explore the analytical methods of isotope and mineral element fingerprints for traceability of tobacco and to find element indicators that characterize regional characteristics. In this paper, Fisher's Stepwise Discriminant Analysis is used to construct the origin traceability model. 9 mineral elements, 4 Sr and Pb isotopes ratios in the tobacco were introduced into the discriminant model. The essential variables were screened out for modeling after the Fisher's Stepwise Discriminant Analysis, such as group mean equality test, covariance matrix, step statistics, and eigen values tests. The homogeneity testing results of the group's average showed that there were significant differences in 9 indexes of tobacco, such as Cu, Zn, Cr, Ni, Cd, Pb, $^{87}Sr/^{86}Sr$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$, among different countries (see Table 1 and 2 for F values differences between groups). Among them, $^{87}Sr/^{86}Sr$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$ have the most obvious differences, and their F values reach 122.238, 161.295, and 129.181, respectively, which is particularly effective in distinguishing the origin. The 5 discriminant functions formed by these 9 indicators, these functions variances, accounted for 70.0%, 18.5%, 8.9%, 2.2%, and 0.4% of the total variance, respectively, and the cumulative variance accounted for 100% of the total variance. Based on the above, the discriminant function of tobacco origin for 6 countries was finally constructed (see Table 3). In practical application, the

Table 2: The Strontium and lead isotope ratio in the tobacco leaf from different countries.

Nation	Statistical indicator	$^{87}Sr/^{86}Sr$	$^{208}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{206}Pb/^{204}Pb$
Argentina ($n = 12$)	Ave.	0.71522^{b}	38.318^{d}	15.609^{c}	18.322^{d}
	S.D.	0.00038	0.184	0.012	0.121
	Min.	0.71465	38.024	15.596	18.148
	Max.	0.71585	38.641	15.635	18.537
Brazil ($n = 10$)	Ave.	0.71559^{b}	38.746^{bc}	15.639^{bc}	18.703^{bcd}
	S.D.	0.00115	0.124	0.013	0.110
	Min.	0.71445	38.552	15.623	18.550
	Max.	0.71802	38.963	15.665	18.817
Zimbabwe ($n = 17$)	Ave.	0.74148^{a}	41.122^{a}	16.450^{a}	22.388^{a}
	S.D.	0.00586	0.789	0.223	1.187
	Min.	0.73248	39.881	16.078	20.451
	Max.	0.75604	42.420	16.913	24.574
U.S. ($n = 6$)	Ave.	0.71334^{b}	38.578^{cd}	15.638^{bc}	18.737^{bc}
	S.D.	0.00231	0.127	0.021	0.140
	Min.	0.71051	38.421	15.615	18.560
	Max.	0.71691	38.742	15.671	18.871
Zambia ($n = 8$)	Ave.	0.73999^{a}	38.891^{b}	15.699^{b}	18.810^{b}
	S.D.	0.00464	0.239	0.021	0.175
	Min.	0.73405	38.706	15.659	18.553
	Max.	0.74644	39.450	15.734	19.158
China ($n = 28$)	Ave.	0.71573^{b}	38.563^{cd}	15.652^{bc}	18.455^{cd}
	S.D.	0.00495	0.128	0.018	0.082
	Min.	0.70965	38.372	15.620	18.309
	Max.	0.72567	38.846	15.701	18.610
Total ($n = 81$)	Ave.	0.72326	39.120	15.815	19.347
	S.D.	0.01266	1.115	0.345	1.674
	Min.	0.70965	38.024	15.596	18.148
	Max.	0.75604	42.420	16.913	24.574
Significant difference between regions	p value	0.000	0.000	0.000	0.000
	F value	122.238	116.015	161.295	129.181

* Different uppercase characters in each column indicate significantly different mean values according to Duncan test ($p < 0.05$).

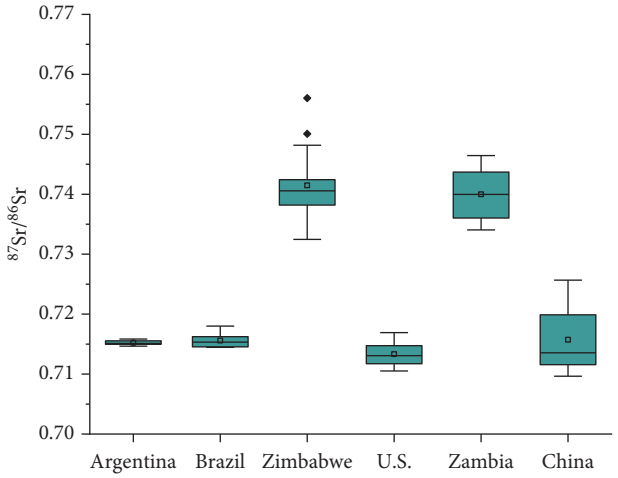

Figure 10: The box plots of $^{87}Sr/^{86}S$ isotope ratio.

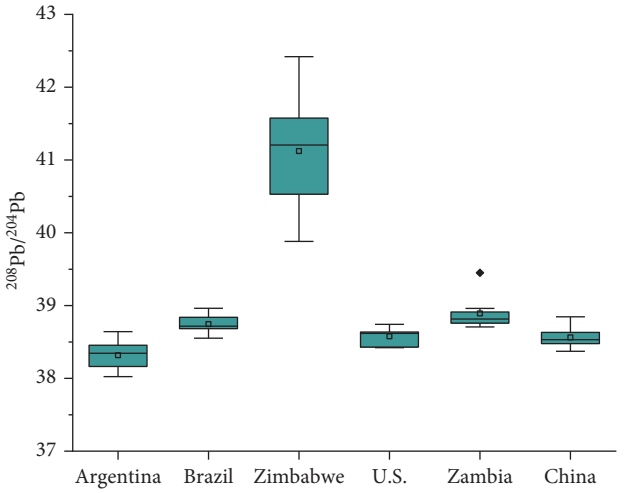

Figure 11: The box plots of $^{208}Pb/^{204}Pb$ isotope ratio in tobacco leaf from different countries in tobacco leaf from different countries.

Cu, Zn, Cr, Ni, Cd, Pb, $^{87}Sr/^{86}Sr$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$ values obtained from blind samples were substituted into the discriminant functions of the six countries, and the blind samples could be classified into the region with the largest function value.

Many researches have shown that stable isotope ratios and mineral elements, combined with multivariate statistical analysis have proven to be particularly useful in tracing the geographical origin of the crops [3, 33–35]. These tracers

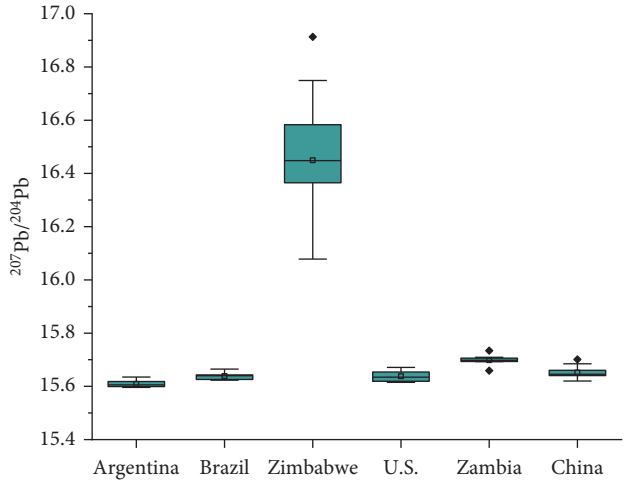

Figure 12: The box plots of $^{207}Pb/^{204}Pb$ isotope ratio.

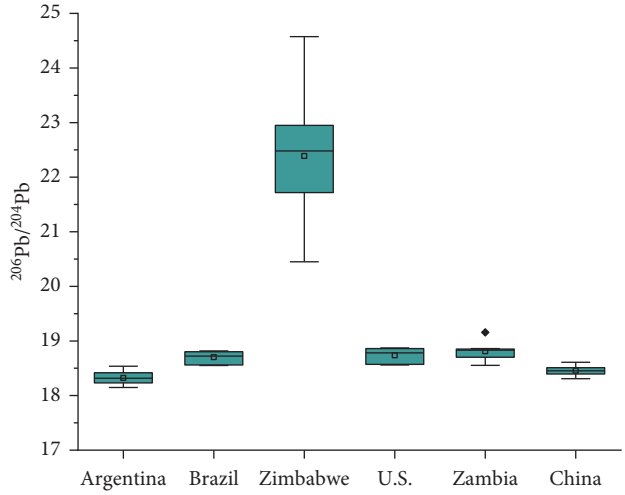

Figure 13: The box plots of $^{206}Pb/^{204}Pb$ isotope ratio in tobacco leaf from different countries in tobacco leaf from different countries.

Table 3: Fisher's linear discriminant classification function coefficients.

Inspection indicator	Geo-origin					
	Argentina	Brazil	Zimbabwe	U.S.	Zambia	China
Cu	28.288	28.227	29.283	28.364	27.952	29.015
Zn	−3.708	−3.597	−3.637	−3.411	−3.795	−3.657
Cr	87.133	81.982	87.466	85.833	79.718	80.827
Ni	33.708	33.702	34.240	32.896	34.519	35.149
Cd	170.098	170.210	172.580	169.258	171.664	173.317
Pb	−144.816	−145.781	−147.953	−145.185	−147.478	−144.015
$^{87}Sr/^{86}Sr$	57461.043	57441.360	59063.574	57222.249	58924.132	57584.022
$^{207}Pb/^{204}Pb$	21926.290	21864.961	22157.672	21854.720	21950.363	21983.502
$^{206}Pb/^{204}Pb$	−3880.732	−3868.292	−3908.523	−3865.970	−3884.012	−3890.509
Constant	−156198.966	−155454.980	−160517.669	−155189.094	−157574.881	−157015.224

reflect the characteristics of the soil, climate, fertilizer, processing, and storage of the crops [1–4].

In the origin discriminant functions, the absolute value of the coefficient reflects the element weights in the origin determination [3, 34]. The larger the absolute value of the coefficient, the greater the impact on the origin determination. From Table 3, the absolute value of the coefficients of $^{87}Sr/^{86}Sr$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$ are absolutely bigger than the others. This indicates that these three isotope ratios play a key role in the traceability of the

Table 4: The validation of fisher's linear discrimination functions.

Authentication method	Statistics	Geo-origin	Predicted group membership						Total
			Argentina	Brazil	Zimbabwe	U.S.	Zambia	China	
Initial verification[a]	Discriminate number	Argentina	12	0	0	0	0	0	12
		Brazil	0	10	0	0	0	0	10
		Zimbabwe	0	0	17	0	0	0	17
		U.S.	0	0	0	6	0	0	6
		Zambia	0	0	0	0	8	0	8
		China	1	0	0	0	0	27	28
	Discriminate accuracy%	Argentina	100.0	0.0	0.0	0.0	0.0	0.0	100.0
		Brazil	0.0	100.0	0.0	0.0	0.0	0.0	100.0
		Zimbabwe	0.0	0.0	100.0	0.0	0.0	0.0	100.0
		U.S.	0.0	0.0	0.0	100.0	0.0	0.0	100.0
		Zambia	0.0	0.0	0.0	0.0	100.0	0.0	100.0
		China	3.6	0.0	0.0	0.0	0.0	96.4	100.0
Cross-validation (leave a law)[b]	Discriminate number	Argentina	12	0	0	0	0	0	12
		Brazil	0	10	0	0	0	0	10
		Zimbabwe	0	0	17	0	0	0	17
		U.S.	0	0	0	6	0	0	6
		Zambia	0	0	0	0	8	0	8
		China	3	0	0	1	0	24	28
	Discriminate accuracy%	Argentina	100.0	0.0	0.0	0.0	0.0	0.0	100.0
		Brazil	0.0	100.0	0.0	0.0	0.0	0.0	100.0
		Zimbabwe	0.0	0.0	100.0	0.0	0.0	0.0	100.0
		U.S.	0.0	0.0	0.0	100.0	0.0	0.0	100.0
		Zambia	0.0	0.0	0.0	0.0	100	0	100.0
		China	10.7	0	0	3.6	0	85.7	100.0

a is the total initial determination accuracy is 98.8%. [b] the total cross-discrimination accuracy rate is 95.1%.

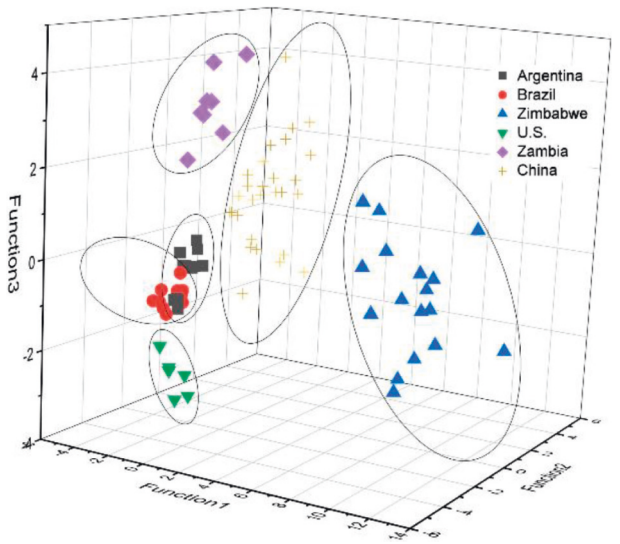

Figure 14: The 3D score plots of tobacco samples on the first three discriminant functions.

origin. The results are consistent with previous reports. In these researches, $^{87}Sr/^{86}Sr$, $^{207}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$ mainly reflect the elemental profile of the soil and vary from region to region, related to the "terroir" of tobacco [33, 35]. As shown in Table 3, the coefficient of Zn and Cu is very small. It indicates that Zn and Cu are not only affected by soil of origin but also related to external impurities in tobacco growing and processing [3, 17–20, 34]. In tobacco growing and processing, Zn is an essential trace element of tobacco. Zinc fertilizer needs to be applied in the process of tobacco planting. And, a Bordeaux mixture prepared with $CuSO_4$ is commonly used in plant fungicides.

The initial verification and a cross-verification method validate the model in identifying tobacco production, as shown in Table 4. In the initial verification results, all tobacco leaves from Argentina, Brazil, Zimbabwe, the United States, and Zambia were correctly classified, and the initial discrimination accuracy was 100%. One Chinese tobacco leaf was misclassified as Argentina, and the initial discrimination accuracy was 96.4%. In the cross-validation results, tobacco leaves from Argentina, Brazil, Zimbabwe, the United States, and Zambia were all correctly classified, and the cross-discrimination accuracy rate was 100%. 4 Chinese tobacco leaves were misclassified (3 from Argentina and 1 from the United States). The cross-discrimination accuracy rate is 85.7%. In conclusion, based on Fisher's stepwise discriminant analysis, the initial determination accuracy rate of tobacco leaf origin is 98.8%. The cross verification accuracy rate is 95.1%, which can realize the origin identification of most tobacco leaves. The first three discriminant function scores of the tobacco leaf samples are plotted as a scatterplot (Figure 14). It can be intuitively seen that tobacco leaves from different countries have their own spatial distribution characteristics, and Argentina and Brazil have some crossovers.

4. Conclusions

In this study, the contents of 9 mineral elements and the ratios of 4 strontium and lead isotopes in 81 tobacco leaf

samples from 6 countries including Argentina, Brazil, Zimbabwe, the United States, Zambia, and China, were determined. Using variance analysis, the results showed that 6 mineral elements (Cu, Zn, Cr, Ni, Cd, and Pb) had significant differences between different countries ($p < 0.001$, F value ˃7). At the same time, the 4 isotopic ratios of Sr and Pb ($^{87}Sr/^{86}Sr$、 $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$) were more significantly different ($p < 0.001$, F value ˃116). The fingerprints of mineral elements, Sr and Pb isotope ratios in different countries have specific regional characteristics. Among them, the content of mineral elements in Zambian tobacco leaves is generally low, and four elements such as Zn, Cr, As, and Cd are not detected. In Zimbabwe tobacco, the average values of $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$ are much higher than in other countries and do not overlap with the other tobacco. Through Fisher's stepwise discriminant analysis, the effective identification of the origin of tobacco leaves is realized via 9 characteristic traceability indicators (Cu, Zn, Cr, Ni, Cd, Pb, $^{87}Sr/^{86}Sr$, $^{207}Pb/^{204}Pb$, and $^{206}Pb/^{204}Pb$). The initial validation and cross-validation accuracy of the function for tobacco leaf origin were 98.8% and 95.1%, respectively. The combination of Sr and Pb stable isotope ratio and mineral element content can effectively discriminate the origin of tobacco leaves, and the discrimination accuracy is very high.

Data Availability

The original data used to support the findings of this work can be obtained from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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