Abstract

The aim of this work was to develop an analytical methodology based on chemical fingerprint to trace the geographical origin of marijuana samples dealt in the city of São Paulo. Nearly 150 samples seized in three main cannabis producing regions in Brazil were analyzed for inorganic constituents using sector field inductively coupled plasma mass spectrometry and stable isotopes ratios of carbon and nitrogen through an isotope ratio mass spectrometer. A way to classify samples of unknown origin was established using linear discriminant analysis. The most important parameters to discriminate groups were carbon and nitrogen isotopic ratios, and the levels of copper, cobalt, barium, lanthanum, zinc, iron, yttrium and manganese. Using this model, 80 samples seized in São Paulo, a most important consumption center, had their geographical origin estimated. The results show that most of them turn up from the Paraguai-Mato Grosso do Sul route. An interesting product of this work was to demonstrate the possibility of using seized samples to create a national databank for monitoring the geographical origin of marijuana consumed in the country.

Introduction

Nowadays, the consumption and production of drugs involves almost all countries, moving about US$ 500 billions per year around the world. According to 2004 United Nations Office on Drugs and Crime (UNODC) report, 3% of the worldwide population (185 millions of people) or 4,7% in the 15 to 64 year-old population bracket use illicit drugs[1]. Marijuana or *cannabis* reaches about 150 millions users, with a productivity from 30,000 a 32,000 tons a year. Amphetamine, cocaine and heroin users are about 30, 13 and 9 millions of people[1].

South America is one of the major producer, dealer and consumer points of illicit drugs. Originally, Brazil was conceived as a classical transit country for cocaine produced in Colombia and Bolivia. However, in the last decades this picture has been changed and Brazil became a significant producer of narcotics moving about 10 billion dollars a year[1]. The efforts to combat drug trafficking allied to the rise in production, place Brazil in fourth in quantities of marijuana apprehension while Mexico, USA and Nigeria lead this rank[1].

Despite of this problem, detailed studies regarding to production, dealing and consumption of this drug in Brazil are still scarce. Official data provided by the Brazilian Federal Police Department (DPF) suggest that the main producing zones in the country are located in the Northeastern region, more specifically in the semi-arid zone between Pernambuco and Bahia States, known as the Marijuana Polygon, and in the Midwestern region, along the Brazil-Paraguayan border[2]. According to latest UNODC reports, Paraguay is second to Colombia in *cannabis* production in South America[1,3].

The most important Brazilian consumer markets are located in the country's South and Southeast, including the São Paulo city that leads the figures of apprehensions related to the consumption[4]. According to the Brazilian Federal Police and UNODC information, this marijuana consumed in the city is not produced locally but comes from the producing zones mentioned above[5,6]. By the end of the 90's, Marijuana Polygon appears as the main

cannabis supplier to São Paulo State[4]. However, recent data indicate Paraguay as the main supplier to the country and that can be related to a change in the traffic routes as a consequence of the intensive and sustained field raids in the Northeast region since 1999. Despite of that, there is no reliable information to verify the truthfulness about routes of traffic.

The use of chemical fingerprint has escalated in order to set a classification pattern that can match drug samples to their geographical origin. This chemical fingerprint can be established using organic[7-8,9,10], inorganic[7,11-.12.13,14,15,16, 1718] or isotopic[19-20,21,22,23] profiles of the samples which are related to the plant growth conditions such as climatic conditions and availability of the elements in the soil. These different profiles can be used jointly to obtain complementary information about the origin of the samples, which make it a distinct feature[18,24]. Despite of the interest about this subject, these works are hindered by the lack of samples of well-known origin that can be used as reference to source the origin of unknown. Moreover, there is no information about the implementation or the effective use of these techniques to track the traffic route of an important drug-dealing center as in the case of the city of São Paulo.

Generally, the plants inorganic profile reflects the availability of elements in the soil. This feature allows the using of plant samples as environmental pollution biomonitor, hence they can appear as element accumulator, mainly toxic metals[25,26]. There are many articles in the literature that show the feasibility of using these parameters to source agricultural materials such as wine[17], wheat[18], tea[16,27] and barley[24] however data related to *cannabis* samples are still scarce[3,12,14].

Coffman and Gentner had shown that *cannabis* plants cultivated in acidic soils present higher absorption for Mn, Fe and Zn[7]. Lately, the same authors evaluated the chemical composition (nutrients and cannabinoids levels) of *cannabis* samples cultivated using mineral fertilizers. It was observed a contrasting concentrations for some elements

for the different analyzed tissues[8]. Although some correlations were noted, the data gathered did not prove conclusive to track the geographical origin of these samples due to the large quantity of related parameters.

Landi uses the atomic absorption and linear discriminant analysis to classify samples separated in inflorescences and leaves from different regions of Italy, based on analysis of nutrient elements[13]. Although it has been possible to classify 100% of the inflorescences samples, according to the author the methodology did not prove itself potentially viable in the analysis of real samples due to the complexity of the problem[13].

The use of inductively coupled plasma mass spectrometry (ICP-MS), a more sensitive technique, allowed Watling to analyze non-essential elements and micronutrients that are present in lower concentrations in plants[15]. About 45 elements were qualitatively measured in *cannabis* seized in Australia, and the most relevant ones to discriminate samples from different origins were rare earth elements (REE), Au, Th, U e W. Some hydroponics *cannabis* were also analyzed and their results are extremely concise with low dispersion rates[15].

Unlike inorganic profile, the stable carbon and nitrogen isotopic composition are related to the climate, mainly water availability, nutrients contents on the soil, other than temperature and light intensity[28,29]. These properties can be useful to source their origins helping to delimit the potential producing areas[30,31]. Recently, works that use these parameters in the sourcing of the origin of illicit drugs such as heroin[19], cocaine[20,21] and *cannabis*[23,32-,33,34] have escalated and had shown the correlation of the stable carbon and nitrogen isotopic composition with climates. However for significant differences, the samples must have been grown under very distinct climatic conditions[23,33]. Shibuya et al. proved the potentiality of isotope ratio mass spectrometry (IRMS) in the establishment of the potential regions of cannabis productions in Brazil, clearly separating samples from humid and dry regions[33]. However this technique was not able to identify the origin of

some samples from areas far away from each other that present similar climatic conditions, and in this way, the model presents some overlapping between Midwestern and Northern groups[33]. According to the authors, in this case, additional information such as the inorganic profiles could be used to access a better discrimination[33].

The aim of this work was to develop a model to classify and trace the geographical origin of marijuana samples seized in the São Paulo city, the most important Brazilian consumption center, using the isotope ratio and high sector field inductively coupled plasma mass spectrometry techniques (IRMS and HR-ICP-MS). The linear discriminant analysis was used to evaluate the results. This work was based on samples seized in the street and has been performed in collaboration to São Paulo State Police Department.

Experimental

Samples

All samples analyzed in this work were seized by the State law enforcement officers and forwarded to the Instituto de Pesquisas Energéticas e Nucleares (IPEN) by the Institutes of Criminalistic (IC) of each State. In order to establish a classification model for the marijuana samples from the city of São Paulo, 144 samples seized in the 3 different Brazilian regions that present the highest levels of seizures and eradications were analyzed (see Table 1). According to the law enforcement officers these samples were cultivated near the regions that they were seized.

Table 1: Brazilian main producing zones and total of samples analyzed.

State	Symbol	Region	Status	Samples
Mato Grosso do Sul	MS	Midwest	Producer and transit State for Paraguayan *cannabis*	30
Pernambuco	PE		Producer	46
Bahia	BA	Northeast	Producer	20
Maranhão	MA		Producer	12
Ceará	CE		Local producer	16
Pará	PA	North	Producer	20
São Paulo	SP	Southeast	Consumer	80
Total				224

Materials and methods

The stable isotope analysis were performed by a continuous flow isotopic ratio mass spectrometry (CF-IRMS), employing a Carlo Erba CHM 1110 elemental analyzer coupled to a Thermo-Finnigan Delta Plus mass spectrometer. About 1 to 1.5 mg of samples was weighted into tin capsules and in the analyzer this organic matter is converted into gases by full combustion, generating N_2 and CO_2. These gases are chromatographically separated and carried by an ultrapure helium flow stream to the mass spectrometer. The $^{15}N/^{14}N$ and $^{13}C/^{12}C$ isotope ratios are evaluated after separation of molecules according to isotopic masses, and finally compared to the calibrated gases ratios using international standards as reference: N_{2Atm} and PDB (Pee Dee Belemnite, standard for C that presents R = $^{13}C/^{12}C$ = 0.0112372), respectively. Results are expressed in relative deviations of the isotope ratios as compared to the standards:

$$\delta X_{sample}(^0/_{00}) = \left[\frac{R_{sample}}{R_{s\tan dard}} - 1 \right] * 1000 \, ,$$

where R is the $^{15}N/^{14}N$ or $^{13}C/^{12}C$.

The evaluation of the methodology was performed using atropine ($C_{17}H_{23}NO_3$), an IAEA (International Atomic Energy Agency) working standard that presents recommended values for $\delta^{13}C$ and $\delta^{15}N$, 28,49‰ and -26,28‰ respectively.

The measurements of inorganic constituents were carried out using a sector field inductively coupled plasma mass spectrometry HR-ICP-MS, Element 1, Finnigan MAT. The wet digestion was performed using a closed vessel microwave oven (MARS 5, CEM CO., model HP-500).

Initially 39 elements were analyzed (Ag, Al, Au, Ba, Be, Bi, Cd, Ce, Co, Cr, Er, Eu, Fe, Ga, Hf, Ho, In, La, Li, Lu, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Sn, Sm, Sr, Th, Tl, Tm, U, V, Yb, Zn) but some on them (such as heavy rare earth elements, Ag, Cd, Au, In and Sc) were below the quantification limits, reducing the methodology to 19 elements. The measured isotopes were listed in Table 2. Indium and Sc were used as internal standard for elements in low (m/Δm = 300) and medium resolution (m/Δm = 3000), respectively. The methodology was evaluated using a standard reference material NIST SRM 1547 (peach leaves), which was analyzed in hexaplicate. For Sr, In and Ba the isobaric interferences were corrected using mathematical equations available in the Element software as listed in Table 3. It was not observed significant interferences for ^{59}Co in low resolution. The HR-ICP-MS experimental parameters were listed in Table 4.

Table 2: Isotopes, resolution and internal standard used in HR-ICP-MS analysis.

Isotopes	Internal standard	Resolution
^{27}Al, ^{59}Co, ^{85}Rb, ^{87}Sr, ^{89}Y, ^{95}Mo, ^{138}Ba, ^{139}La, ^{140}Ce, ^{141}Pr, ^{143}Nd, ^{208}Pb, ^{232}Th, ^{238}U	In	300
^{69}Ga, ^{65}Cu, ^{66}Zn, ^{56}Fe, ^{55}Mn	Sc	3000

Table 3: Isotopes measured by HR-ICP-MS that presented isobaric interferences and respective equations of correction.

Isotope	Equation of correction
^{87}Sr	-0,3857*Rb85
^{115}In	-0,0149*Sn118
^{138}Ba	-0,0028*Ce140-0,0009*La139

Table 4: HR-ICP-MS instrumental settings and data acquisition parameters.

Cone and skimmer	Níquel
Autosampler	CETAC 500 AX
Nebulizer chamber	Scott
Nebulizer	Meinhard
Gas flow (L min^1)	
Sampling	1.2
Auxiliar	0.8
Cooling	16.0
HR-ICP-MS parameters	
Runs and passes	3 x 10
RF power	1250 W
Resolution (m/Δm)	300 e 3000
Segment duration	3 ms
Scan	Escan
Sensibility for ^{115}In	80,000 cps μg^{-1} kg

Sample preparation

The samples were cleaned in sonicator for about 30 minutes in de-ionized water (twice), dried at 40°C for about 24 hours, and ground in an electric mill with ceramic mortar and pestle.

The wet digestion was based on the work of Baffi et al.[35]: about 250 mg of material was weighted in a teflon PFA tubes (HP-500, 100 mL), and then were added 7 mL of HNO_3 65% (40% m/v, suprapur, Merck), 1 mL H_2O_2 (30%, analytical grade, Merck) and 200 μL of HF (40%, suprapur, Merck). The calibration solutions were prepared from SPEX solutions (1 000 μg g^{-1}) in 2% HNO_3.

Data analysis

The assessment of the results and the building of a model to classify unknown samples followed the linear discriminant analysis (LDA), using SPSS (Statistical Package for Social Science) program, version 10.0.5. This method identifies a linear combination of quantitative predictor variables that best characterizes the difference among known groups (called calibration set). Linear discriminant analysis enhances the separation of the groups, allows a classification of unknown samples and lists the group in which each case is most likely a member, and also the probability for belonging to this group. The enter independent together method builds the model using simultaneously all disposable variables while stepwise method selects the most relevant ones, reducing the data set[36]. These new variables are called canonical variables. The first canonical variable account for a large proportion of the variability within the original data and the plot is defined, so that the most significant differences among the groups are displayed along the horizontal axis. The second canonical variable represents the maximum dispersion in a perpendicular direction to the first one, and so forth.

Results and discussion

IRMS analysis

The accuracy and reproducibility for δ^{13}C and δ^{15}N for atropine may be observed in Table 5.

Table 5: Results for atropine (n=6).

	δ^{13}C (‰)	δ^{15}N (‰)
Mean	-26,26 ± 0,28	-28,65 ± 0,13
Recommended value	-26,28	-28,49

The results presented by Shibuya et al. for stable carbon and nitrogen isotopes were summarized in Table 6. The isotopic profiles for samples from dry (Pernambuco, Bahia e Ceará) and humid regions (Maranhão, Pará e Mato Grosso do Sul) were clearly different, indicating that these variables can be used to obtain information regarding the climate of the producing zones. The high dispersion rate for δ^{13}C for the Maranhão group can be explained by the peculiar geographical situation of its Western expanse, whose climate and vegetation are similar to those of the Northern region (the Amazon region), whereas its Eastern area presents a semi-arid climate[33].

Table 6: δ^{15}N and δ^{13}C mean values for *cannabis* samples from the main Brazilian producing zones.

State	N	δ^{15}N (‰)	δ^{13}C (‰)	Climate
NORTHEAST				
Pernambuco	46	1,9±2,9	-26,6±1,1	dry
Bahia	20	1,9±2,5	-26,0±1.2	dry
Maranhão	12	2,9±2,5	-28,8±1,6	humid (W) and dry (E)
Ceará	16	1,7±1,6	-26,6±0,8	dry
MISWESTERN				
Mato Grosso do Sul	30	6,6±1,1	-28,7±1,3	humid
NORTE				
Pará	20	5,0±1,3	-30,3±0,7	humid

Despite the existing information about highly irrigated *cannabis* cultures in Pernambuco and Bahia, no overlapping was observed between these groups and samples from more humid regions. On the other hand, the high dispersion rate observed for δ^{15}N especially for samples from Pernambuco indicates *cannabis* cultures throughout, from the semi-arid region to areas around the São Francisco River Basin and the woodlands (*Zona da Mata*), as well as utilization of fertilizers[33]. Four samples seized in this State presented δ^{15}N values above 8‰, which may be associated to the use of organic fertilizers (such as animal manure). For this reason, they were not taken into consideration in the construction of the final classification model.

Before evaluating the elemental profile of the samples, the information obtained through the IRMS technique was utilized in regrouping the samples and in defining the

calibration set, as per Table 7. Such an evaluation is important, since the temperature and humidity bear a significant influence upon the soil's physical and chemical properties, which directly affect the chemical reactions occurring therein, defining besides its pH, also the availability of nutrient elements and the ion predominant in the soil solution[37]. Generally speaking, soils formed in tropical climates present high weathering rates as compared to those under temperate climates[37]. The more humid and warmer the climate, the stronger the mineral leaching, once heavy rainfall leaches basic ions such as calcium and magnesium and replaces them with acidic ions such as hydrogen and aluminum, such as for example that of the Amazon Region[38]. On the contrary, soils from arid regions tend to become alkaline, once rainfall is not heavy enough to leach basic ions[37].

Table 7: samples regrouped according to the IRMS results. These groupings were used as calibration set in the building of the model to classify unknown samples.

Groupings according to IRMS results	State	Samples
Region 1	Mato Grosso do Sul	30
Region 2 (Marijuana Polygon)	Bahia	20
	Pernambuco	42
	Ceará	16
	Maranhão dry	7
Region 3 (Amazon)	Maranhão wet	5
	Pará	20

HR-ICP-MS

The results for SRM NIST 1547 indicated good accuracy, with a recovery better than 90% for the certified elements. Quantification limits, defined as 10σ (n=8) ranges from 0,02 (for U) to 12 ng g^{-1} (for Zn).

The results for *cannabis* samples are listed in Table 8 jointly with the data obtained from literature where by comparison the Brazilian samples presented high Fe, Sr and Ba levels and low concentrations of Mo, Cu and Zn together with literature available data[7,8].

Table 8: Range of concentration for *cannabis* samples from three main Brazilian producing zones and data from the literature. Mean values are presented in parenthesis.

	Range of concentration					
	Results from this work ($\mu g\ g^{-1}$)			Values from literature ($\mu g\ g^{-1}$)		
Element	Region 1 – MS	Region 2 – Marijuana Polygon	Region 3 – Amazon	Coffman and Gentner[7]	Coffman and Gentner[8]	Landi[13]
Co	0,08 – 2,1 (0,7)	0,06 – 2,5 (0,6)	<0,02 – 0,25 (0,08)			
Cu	5,7 – 69 (22)	1,3 – 36 (9,8)	0,8 – 19,5 (7,7)	7 – 18	67 – 145	10 – 31
Fe	446 – 7660 (2286)	110 – 1600 (785)	132 – 664 (341)	84 – 216	54 – 127	141 – 274
Mn	214 – 622 (361)	175 – 1386 (652)	69 – 2066 (350)	76 – 199	226 – 1767	24 – 93
Mo	0,06 – 2,4 (0,8)	0,08 – 2,1 (0,7)	0,04 – 2,3 (0,6)			0,74 – 2,69
Zn	25 – 284 (85)	23– 121 (56)	13 – 108 (51)	71 – 312		27,5 – 59
Rb	1,2 - 20,7 (7,3)	0,4 – 14,5 (4,8)	0,6 – 18 (5,3)			
Sr	17 – 662 (246)	24 – 406 (152)	76 – 380 (229)	9 – 85		
Ba	46 – 671 (208)	10 – 361 (71)	5,4 – 393 (101)	9 – 77		
Al	384 – 5982 (1427)	98 – 5858 (3774)	106 – 1545 (683)			
Ga	0,06 – 8 (2,5)	0,04 – 2,7 (0,7)	0,04 – 1,2 (0,5)			
Pb	0,1 – 3,1 (0,7)	0,2 – 9,5 (2,8)	0,2 – 4,6 (1,0)			
Y	0,1 – 1,5 (0,57)	0,04 – 1,6 (0,66)	0,009 – 0,2 (0,08)			
La	0,3 – 4,1 (1,3)	0,2 – 7,8 (2,5)	0,008 – 0,7 (0,4)			
Ce	0,4 – 6,1 (1,9)	0,2 – 12 (3,4)	0,2 – 1,0 (0,7)			
Pr	0,03 – 0,7 (0,2)	0,02 – 1,3 (0,4)	<0,02 – 0,09 (0,06)			
Nd	0,3 – 2,3 (1,0)	0,1 – 4,8 (1,6)	0,03 – 0,3 (0,27)			
Th	<0,06 – 0,8 (0,17)	<0,06 – 2,3 (0,4)	<0,06 – 1,2 (0,15)			
U	<0,02 - 0,14 (0,055)	<0,02 - 0,36 (0,09)	<0,02 – 0,08 (0,03)			

Nutrient contents in samples from Region 1 (Mato Grosso do Sul) were high, mainly for Cu and Fe. Aluminium, Ga and Ba levels were also higher than those in the other groups (Table 8). Most soils of this region are of volcanic origin (magmatic rocks, most of them pholeiitic basalt), naturally acidic soils with excess Al. Iron and Mn, also are abundant element in basalt-derived soils, such as this case and Fe can be reach toxic levels according the soil's pH (pH<5.0)[39].

In comparison to the literature data, Cu and Zn concentrations were similar to those obtained by Landi[13], although lower than those presented by Coffman and Gentner[8]. It is well known that Zn deficiency is the most generalized and critical in those regions and probably relates to the composition of the parent material[39]. The Mo value in *cannabis* samples was also lower than those available in the literature, which may point out to a deficiency of this element in the soil[39].

Samples seized in Region 2 (the Marijuana Polygon) presented high levels of U, Th, Pb, Mn and rare earth elements (REE), mainly lanthanum and cerium. The soils of that region are in largely formed of granitic rocks and granulites[40], which are naturally lanthanides-enriched (in some cases extremely enriched)[41,42]. The levels of Zn and Mo nutrients are similar to those observed for Regions 1 and 3, the Zn level below the values described in literature (Table 8), whereas the Mn average appeared above those of the other groups. According to Horowitz and Dantas, Mn concentrations are high in the Northeastern dry backcountry (*Sertão*) probably as a consequence of low precipitation rates, low content of organic matter and relatively low pH[41]. Copper, nevertheless, presented considerably lower values than those of Region 1. The deficiency of that element is well known, notably its absorption by plants inhibited by high Fe concentrations, current in that region[37,43]. Leon finds that that the soils in the dry backcountry of the Northeast (*Sertão*) apparently do not present Mn deficiencies nor do those of the semi-arid

region (*Agreste*) appear to present Zn and Mo deficiencies[44]. Variability of those elements in the Northeastern samples was large, and may indicate that they were produced not only in the Pernambuco dry backcountry (*Sertão*), but also in others with lower deficiency of nutrients, such as the semi-arid region (*Agreste*) and the woodlands (*Zona da Mata*).

The concentrations of Cu, Mo and Zn nutrients obtained for Region 3 (Amazon) samples were similar to the levels reported for Region 2. The samples seized in Region 3 also showed low concentrations of cobalt (below 100 ng g^{-1}), whereas the average arrived at for this element in samples coming from other regions was above 600 ng g^{-1} (Table 8). The low levels of concentration referring to practically all of the elements in samples from the Amazon Region may be explained by the intense leaching of those soils[45]. Despite the region's great geological diversity[46], the climatic conditions strongly influenced the soil's physical and chemical properties[37], hence the similarity in chemical profile of this group's samples.

Construction of model and classification of samples from São Paulo

Once the relevance of each variable in the group discrimination having been evaluated, a classification model was proposed, based on the linear discriminant analysis (LDA). As already mentioned the samples from calibration set were defined according to the groupings of Table 7.

Using the all set of variables (enter independent together method) 100% of the calibration set are assigned to their correct group. The stepwise selection of variables also allowed a 100% successful classification with the employ of 10 out of the 21 available variables. The cross validation, the procedure in which each case is classified into a group according to the classification functions computed from all the data except the case being classified[36], offered similar results (97.8% overall rate of successfully classified cases), with two samples seized in the Region 2 (Marijuana Polygon) classified as originating from Region 1, and one sample from Region 3 (Amazon) as originating from Region 2. Classification of samples from São Paulo and variables used in this model can be found in Tables 9 and 10.

Table 9: Classification of samples from calibration set and those seized in the São Paulo city using linear discriminant analysis and the variables listed in Table 10.

		PREDICT	Predicted Group Membership - Region			Total
			1,00	2,00	3,00	
Original	Count	Region 1	30	0	0	30
		Region 2	0	85	0	85
		Region 3	0	0	25	25
		São Paulo	66	4	10	80
	%	Region 1	100,0	,0	,0	100,0
		Region 2	,0	100,0	,0	100,0
		Region 3	,0	,0	100,0	100,0
		São Paulo	82,5	5,0	12,5	100,0

Table 10: Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions. The variables are ordered by absolute size of correlation within function.

Structure Matrix

	Function	
	1	2
$\delta^{13}C$	-,633*	,499
$\delta^{15}N$	,551*	,007
Ba	,228*	,213
Mn	-,037*	,017
Cu	,249	,377*
Co	-,009	,329*
La	-,208	,250*
Zn	,149	,238*
Y	,009	,067*
Fe	-,016	,035*

* Largest absolute correlation between each variable and any discriminant function.

The most relevant parameters to discriminate the groups were: $\delta^{13}C$, $\delta^{15}N$, Ba e Mn (Function 1 - F1), and Cu, Co, La, Zn, Y, e Fe (Function 2 - F2). According to these results, the climatic conditions are the most relevant features in defining the differences between samples from the studied regions, followed by the micro and macronutrients as well as the rare earth elements availability in the soil. Scores for each variable in discriminant functions 1 and 2 are listed in Table 10. The total variability explained by those two canonic variables was 100%.

The territorial map and samples from Regions 1, 2 and 3 in the canonical variable plot can be seen in Figure 1, where it may be observed that the discrimination between Regions 1 and 2 is basically related to F1, a parameter associated with climatic conditions;

on the other hand, Region 3 samples can be distinguished from the rest by the fact they present a lower F2 (mostly associated with levels of nutrient elements – Table 10).
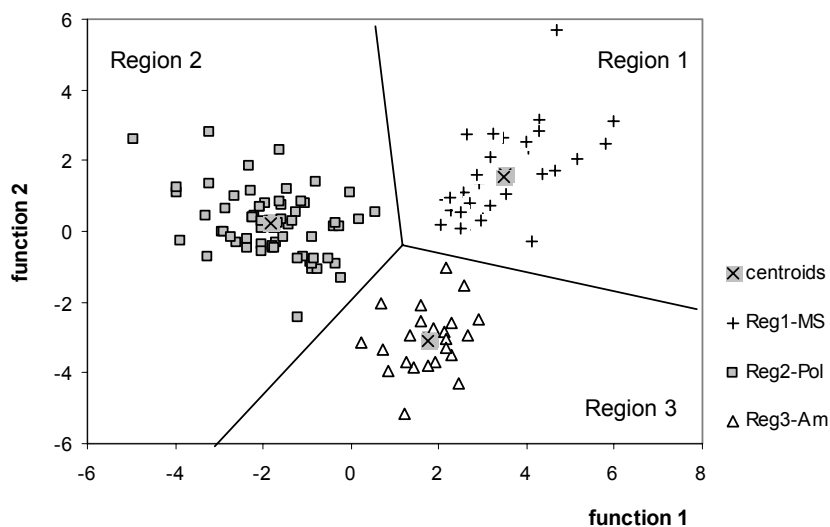

Figure 1: Territorial map established by linear discriminant analysis using the following parameters: $\delta^{13}C$, $\delta^{15}N$, Cu, Co, Ba, La, Zn, Fe, Y e Mn.

The origin of 80 samples seized in the city of São Paulo was estimated from this model forward. Nearly 83% showed chemical profiles similar to those samples seized in Region 1 (Mato Grosso do Sul), whereas 5% appear to be from Northeastern origin (Region 2), and 12,5% from the Amazon Region (Region 3) - Table 9. Although there was no overlapping for the calibration set between Regions 1, 2 and 3, some of the São Paulo samples could not be clearly classified (Group A - Figure 2).
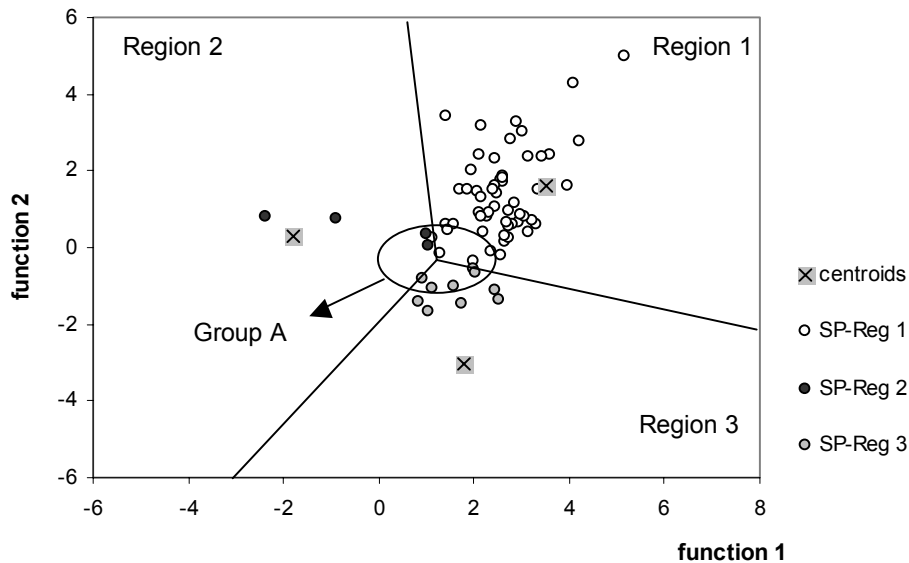
Figure 2: Geographical origin of the samples seized in the São Paulo city according to the LDA model, using the following variables: $\delta^{13}C$, $\delta^{15}N$, Cu, Co, Ba, La, Zn, Fe, Y e Mn.

Figure 3 shows the isotopic profiles of the São Paulo samples. It can be seen that those classified as coming from Regions 1 and 3 present similar profiles, and their origins cannot be determined from the data obtained through IRMS technique. Those results stress the need to apply a large set of variables, which would supply complementary data.

Since LDA was unable to identify a given sample that does not originate from any of the studied regions, the probability cannot be discarded that Group A samples may have come from other regions not taken into consideration by the model (inside or outside Brazil). Nevertheless, this model identified the source of samples seized in streets of São Paulo, indicating that most of them turn up from the Paraguai - Mato Grosso do Sul route.
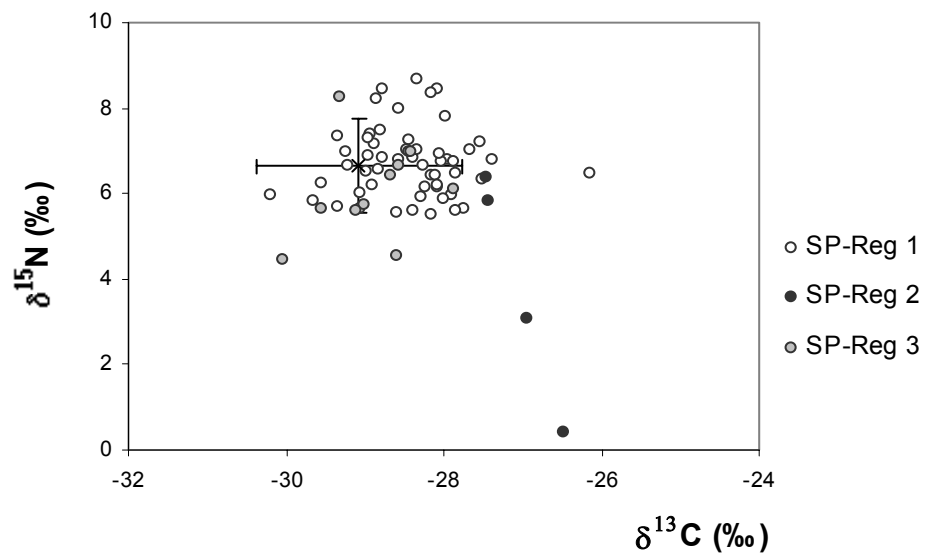
Figure 3: Isotopic profiles of samples seized in the city of São Paulo, and their classification according to LDA model. Bars represent 1σ for Mato Grosso do Sul group.

Conclusions

As previously discussed, the main task in sourcing marijuana is to obtain a reliable database using samples of well-known provenance. The most accepted procedure in cases as such is to use samples eradicated at the plantation site. However, this approach presents obvious inconveniences, apart from the fact that once a region has been identified as producing marijuana, owners relocate their plantations. Therefore, this work has attempted to demonstrate the possibility of using samples seized from drug dealers and users during routine work by law enforcement officers. Such strategy presents several advantages related to security, cost, periodicity and huge quantities of available material.

The IRMS technique has proved itself particularly useful in preliminary evaluation of samples, by clearly separating materials from both humid and dry regions, by subsidizing from that early step forward the whole process of classification. However, in cases where *cannabis* originates from regions of similar climates, the use of additional information concerning the soil geochemistry becomes essential. The linear discriminant analysis is extremely useful to summarize many variables by a few factors and to determine more important parameters in the discrimination of groups, proving itself to be fundamental to the evaluation of results. Although of course to obtain reliable results depends on a full understanding of the problem under consideration and of all variables involved.

Although the number of analyzed samples is small, the results indicate that there is no significant traffic between the Marijuana Polygon and the city of São Paulo. In fact just the opposite: most of the *cannabis* probably proceeds from the Paraguai-Mato Grosso do Sul route, as recently pointed out by the official information. As previously mentioned, that picture may be the end result of the Federal Government's efforts to combat cultures of that herb in the Polygon region.

Still with reference to the samples seized in São Paulo, the results indicate that some would probably originate in the Amazon region, which may suggest that Maranhão has been consolidating its position as a significant producer, with traffic routes to the country's main consumer centers. In that case, it is believed that an analysis embodying a larger number of samples would render more representative and forceful results. This will make feasible a close monitoring of the country's drug market, by taking stock of possible routes, farming conditions and firstly in importance, the rise of new producing regions, thereby alerting the authorities and preventing the establishment of drug chains in local villages.

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